Life History and Reproductive Variation in the Spotted Skink, *Niveoscincus ocellatus* (Gray 1845)

by

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Declaration: This thesis contains no material which has been previously accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the Thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgment is made in the text of the thesis.

signed: 

date: 23/2/98

Abstract

Life history and reproductive variation in the spotted skink, *Niveoscincus ocellatus*

The spotted skink, *Niveoscincus ocellatus* is a widely distributed small to medium size skink (3-12 g) which occurs throughout eastern and central Tasmania in a variety of climatic regimes. This thesis provides the first major ecological study of this species and describes in detail the life history and reproductive characteristics of two populations living at the climatic extremes of the species' distribution: a site on the Central Plateau represented the cold extreme and a site at Orford on the east coast represented the warm extreme.

Niveoscincus ocellatus is a viviparous species that reproduces annually across its range. It shows an asynchronous gonadal cycle, with maximum male gonadal development in late summer and mating from April to June and August to September. Vitellogenesis occurs predominantly in autumn and continues through winter with ovulation in spring (September to October). Parturition occurs in January in the temperate Orford population, and in February in the subalpine Central Plateau population. In addition to minor variations in the timing of reproductive events between years at each site, *N. ocellatus* shows a degree of flexibility in the timing of reproductive events between sites. Ovulation and parturition occur approximately one month later at the Central Plateau, but there is little difference in the length of the gestation period between sites. An ability to show flexibility in the timing of reproductive events is undoubtedly responsible, in part, for the widespread distribution of *Niveoscincus ocellatus* in a range of climatic conditions.

Niveoscincus ocellatus displays considerable geographic variation in life history traits. Maturity is delayed at the Central Plateau (3 years)

compared to Orford (2 years). As a result, individuals from the Central Plateau mature at a large size and have a larger maximum size. Associated with their larger size and delayed maturity, Central Plateau females produce larger (mass and number of young) clutches than those from Orford. There is no trade-off between clutch size and size of offspring between populations, and females from the Central Plateau produce more (size adjusted) and larger offspring than females from Orford.

Through a series of experiments I show that life history traits of *N. ocellatus* are phenotypically plastic, and that observed differences in life history traits between sites, and annually within sites, may be explained, at least in part, by phenotypic responses to environmental variables. For example, growth rate of juveniles is positively correlated to basking opportunities in the laboratory and to thermal conditions in field enclosures, but is not influenced by any underlying genetic differences between populations. Offspring growth rate and phenotype are also influenced by other proximate factors, including the basking behaviour of their mothers prior to parturition in the laboratory. Phenotypically plastic responses allow a flexible approach to life history and reproductive strategies and are of adaptive significance to species such as *N. ocellatus* that occupy a wide geographic and/or climatic range.

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Contents

Chapter 1

General introduction

1.1 General overview

This thesis presents a comparative study of two populations of the Tasmanian spotted skink, *Niveoscincus ocellatus*. The study concentrates on the differences observed in life history patterns of two populations at the climatic extremes of the species' distribution, and investigates possible sources of variation in the observed phenotypes and the consequences of these differences.

The thesis is presented in four sections:

Section 1

The general introduction (Chapter 1) outlines the relevant life history theory, and the current knowledge base concerning geographic variation in life history characteristics, specifically associated with lizards. This section also provides the context for the study, and introduces the knowledge base on Australian reptiles, including Tasmanian species, with emphasis on the family Scincidae. Chapter 2 describes the two study sites, Orford on the East Coast of Tasmania (Orford study site) and Lake Augusta on the Central Plateau (Central Plateau study site) and the reasons for their selection.

Section 2

This section details the observed differences between populations of *N. ocellatus* from the two study sites and includes chapters dealing with the reproductive cycle (Chapter 3), and observed phenotypic differences

in life history traits (Chapter 4). Annual variation and variation between sites in reproductive output (absolute clutch mass and relative clutch mass), number and size of young, and the relationship between female size and number of young, are discussed (Chapter 4). The final chapter in this section provides data on age and size relationships in *N. ocellatus* from the two study sites using age data obtained by skeletochronology (Chapter 5). There is an emphasis on differences in age and size at maturity between study sites and sexes. From these relationships, growth curves were constructed. Variation in age or size at maturation is of great interest in the study of life history evolution because of the diverse effects of maturation on fitness (Bernardo, 1993).

Section 3

In this section the source of variation of observed differences in life history traits between populations is investigated. Factors influencing growth rate are explored in detail (Chapter 6). Explaining variation in growth rate between populations poses a challenge for studying life history evolution because variation in growth rate has direct consequences for many other life history traits, including age at maturity, adult body size and fecundity (Niewiarowski, 1995). Several lines of investigation are used in this chapter. An initial laboratory study of the effect of basking opportunity on juvenile growth rate is presented in which differences in the phenotypic response of growth rate, together with any underlying genetic differences between populations, was examined. A second experiment is described in which growth rates of juveniles in large field enclosures located in different thermal environments was investigated. Differences in growth rates associated with environment, population, sex, and size of young were analysed. Chapter 7 investigates sources of variation in juvenile phenotype. Maternal basking opportunity is identified as an important source of variation and its effects on growth rate are further investigated. The final chapter in this section (Chapter 8) examines the cost of pregnancy, through its effect on maternal sprint speed, within and between populations.

Section 4

The final chapter in this thesis (Chapter 9) provides a general discussion of the observed differences in life history traits between the two populations of *N. ocellatus* studied, and makes some suggestions concerning work that would aid in our understanding of geographic variation within species.

1.2 Life history theory

An extensive literature on the theory of life history evolution has developed in the last two decades (see Stearns (1976; 1977) for early reviews and Stearns (1992) and Roff (1992) for more recent reviews). Life history theory attempts to explain patterns of variation observed in nature, and to predict the relationship between life history and the environment (Ballinger, 1983).

Life history tactics were defined by Stearns (1976) as a series of co-adapted traits "designed by natural selection to solve a particular ecological problem". Life history theory makes the simplifying claim that the phenotype consists of demographic traits: birth, age, and size at maturity, number and size of offspring, growth and reproductive investment, length of life, and death, connected by constraining trade-offs between them (Stearns, 1992). Analysis of these interactions explains phenotypic adaptation.

Stearns (1992) proposes eight principal life history traits that are related to reproduction and survival. These are:

1. size at birth,
2. growth pattern,
3. age at maturity,
4. size at maturity,
5. number, size, and sex ratio of offspring,
6. age and size specific reproductive investments,

7. age and size specific mortality schedules, and
8. length of life.

The life history strategy of an organism may be viewed as the culmination of "complex adaptation" and refers to the coordinated evolution of all life history traits together (Stearns, 1992). Stearns (1992) argues that, although early trends in understanding such strategies were to categorise suites of traits into a few general trends, it is now recognised that this is an oversimplification and understanding must be based on an analysis of the evolution of individual traits.

Many organisms have been, and continue to be, used for the study of life history evolution. However, lizards are particularly well suited to such studies because of the interesting variations they exhibit and the ease with which their life history characteristics can be quantified (Ballinger, 1983). As a result, studies of lizard life history evolution have assumed central importance in the testing of life history theory (Howland, 1992).

A central assumption of life history theory is that natural selection acts to maximise fitness via selection on life history traits (Roff, 1992; Stearns, 1976; 1989b; 1992).). If life history traits are co-adapted, the concept of trade-offs is central to the understanding of life history evolution (e.g. McGinley, Temme and Geber, 1987; Dunham, Grant and Overall, 1989; Schwarzkopf, 1994; Olsson and Shine, 1997). Natural selection should therefore produce phenotypes that are compromises between the costs and benefits of changing any particular character (Schwarzkopf, 1994). At least 45 trade-offs between life history traits have been defined (Stearns, 1989b; 1992). Of these, the most studied are those between current reproduction and survival, current reproduction and future reproduction, reproduction and growth, reproduction and condition, and number and quality of offspring.

Stearns (1992) defined several types of trade-offs that are pertinent to the study of geographic variation in life history traits. Microevolutionary trade-offs are defined by the responses of populations to selection, and are the type of trade-offs responsible for observed differences in life history

traits between populations that occur in different areas (Stearns, 1992). For example, selective pressures differ as a result of climatic conditions and may bring about changes in the optimum series of co-adapted life history traits. Microevolutionary trade-offs in life history traits result both from genetic correlations and from their expression and can therefore include a component of plasticity in the expression of the phenotype. Physiological trade-offs are caused by allocation decisions between two or more processes that compete directly with one another for limited resources within a single individual.

1.3 Variation in lizard life history traits

Lizards exhibit a great diversity of life histories (Tinkle, Wilbur and Tilley, 1970; Ballinger, 1983; Dunham, Miles and Reznick, 1988) and the challenge remains to explain the diversity of strategies among species and the geographic variation observed within many species that occupy wide geographic, altitudinal or climatic ranges.

There have been several extensive reviews of the life history of lizards. However, a major shortcoming in lizard ecology remains the limited number of species for which even minimal data are available (Vitt and Pianka, 1994). Tinkle (1969) and Tinkle, Wilbur and Tilley (1970) presented the first extensive summaries of life history traits of lizards and made some predictions based on general lifestyle. Tinkle (1969) presented a review of 112 species of lizards from 53 genera and 10 families which, at the time, represented approximately 4 % of known species. The review used the criterion of reproductive effort as suggested by Williams (1966a, 1966b) to characterise and summarise species.

Lizards were grouped according to their mode of reproduction (oviparous versus viviparous), age at first reproduction ("early" versus "late"), frequency of reproduction (single versus multiple clutches per season), and distribution (temperate versus tropical). Several generalisations were made from the data. For example, species with delayed sexual maturity tend to be larger at maturity and throughout life,

to produce a single large clutch each season, to be either viviparous or oviparous, and are more likely to be found in temperate regions (Tinkle, 1969).

In his review, Tinkle (1969) made important distinctions between long and short lived species, and emphasised the conditions that may favour delayed maturity and long life expectancy. He suggested that in long lived species selection must favour individuals that put less energy and resources into reproduction each season (particularly if fecundity increases with age), because such a strategy increases their lifetime reproductive success. Delayed maturity may occur when:

1. early maturing individuals are selected against because their inexperience at holding a breeding territory makes it difficult or impossible for them to breed;
2. early maturation results in maturity in a season not favourable for reproduction;
3. the cost of rapid development is high post maturity mortality or very low fecundity; or
4. rapid attainment of large size is desirable.

Since these early studies, numerous other medium or long term field-based studies of lizard life histories (mainly on North American oviparous species) have documented variation in life histories between different lizard species and between populations of the same species. These studies have contributed to our understanding of the evolution of life history (e.g. Ballinger, 1973; 1983; Ferguson, Bohlen and Wooley, 1980; Dunham, 1982; Ruby and Dunham, 1984; Jones and Ballinger, 1987; Jones, Ballinger and Porter, 1987; Grant and Dunham, 1990; Tinkle, Dunham and Congdon, 1993).

There have also been a number of recent attempts to review and generalise lizard and snake life history (Stearns, 1984; Dunham and Miles, 1985b; Dunham, Miles and Reznick, 1988). Stearns (1984) stressed that the evolution of life history is strongly influenced by female body size and phylogenetic effects and that adaptation to specific environmental conditions is less important. However, this work has

been criticised (Dunham and Miles, 1985a) because of errors in the data set, and biases towards certain groups and life history strategies (for example only 6 viviparous species were included).

On re-analysis of an expanded and corrected data set, Dunham and Miles (1985b) argued that the within-family patterns of covariation of life history traits may be explained in terms of local adaptation, plasticity of response to local environmental heterogeneity and physiological constraints (predominantly mode of reproduction). Although Dunham and Miles (1985b) found significant variation in life history characteristics such as clutch size and age at maturity at the level of order, family and genus, they still argued strongly that phylogeny is an important correlate of life history.

Dunham, Miles and Reznick (1988) provided the next extensive review of squamate life histories and included data from 185 populations of lizards belonging to 149 species. However, they recognised that their data set was biased (because of the available data) towards iguanids and recommended caution in interpreting their data set for other groups of lizards which were under-represented. Following the work of Vitt and Congdon (1978) and Vitt and Price (1982), Dunham, Miles and Reznick (1988) included data on relative clutch mass (RCM) as an important life history trait and measure of reproductive effort in lizards.

Dunham, Miles and Reznick (1988) provided a generalised summary of life history across all lizard species for which adequate data existed. Lizards tend to mature at an early age (~ 18 months), and at a small body size (62 mm); their mean adult size (72 mm) is only marginally larger than their size at maturity. Most species are oviparous (83%), while the remainder are viviparous (17%). The majority are multiple brooded (65%), the rest are typically single brooded, and biennial reproduction is rare. The mean clutch size is 5.3 eggs/offspring per brood and females have a low relative clutch mass (0.24). This pattern is generally conserved between lizard families. Like Tinkle, Wilbur and Tilley (1970), they found that viviparous species are more likely to be single brooded and late-

maturing, and temperate species are more likely to be single brooded than tropical species.

Most recent interspecific analyses of life history traits have revealed that many of the key traits show strong covariation (e.g. Dunham, Miles and Reznick, 1988; Miles and Dunham, 1992; Bauwens and Diaz-Uriate, 1997). For example, age at first reproduction, clutch size and size of neonates strongly covary with body size in lizards. Large lizards exhibit delayed maturity and produce clutches with large numbers of relatively large offspring (Miles and Dunham, 1992).

Dunham, Miles and Reznick (1988) also provided a valuable comparison of populations within species, and argued that such comparisons often provide a quantification of geographic variation in life history traits. Furthermore, if these comparisons include detailed ecological data, they may offer tests of specific predictions from life history theory. They concluded, however, that the available data from intraspecific comparisons are often not consistent with current life history theory. Their conclusion was that variation in life history is a result of a combination of demography, ecology and phylogeny. More specifically, life history traits are modified by local adaptation (including habitat specificity and environmental predictability), phylogenetic constraint, foraging mode, and allometric covariation among traits.

Despite the accumulating data base of both interspecific and intraspecific studies, the observed life history variation among species and between populations of the same species still cannot be explained satisfactorily (Tinkle and Dunham, 1986). Dunham, Miles and Reznick (1988) argued that more data on a variety of taxa are still required. Complete data on the important life history traits was only available to them for 56 species of lizards, and knowledge of life history variation remains especially poor for several diverse lizard groups including the Scincidae.

1.3.1 Intraspecific variation in life history traits of lizards

Life history characteristics vary widely among species of lizards and populations. Most authors seek adaptive or phylogenetic explanations for life history patterns, which are usually presumed to reflect genetic differences. However, life histories are often phenotypically plastic, varying in response to temperature, food availability, and other environmental factors (Caswell, 1983; Lessells, 1991). Phenotypic plasticity in life history traits is a pervasive phenomenon (Caswell, 1983), which has only recently been incorporated into life history theory (Stearns, 1989a). Phenotypic variation to environmental variables is often continuous, in which case the relationship between phenotype and the environment is called a reaction norm (Lessells, 1991; Stearns, 1992).

Geographic variation in life history phenotypes between populations of a single species is often assumed to reflect genetic divergence caused by natural selection. The relative contribution of genetic and environmental sources of phenotypic variation is rarely determined. However, distinguishing between phenotypic plasticity induced by proximate environmental variation and genetic divergence is fundamental to understanding the ecological and evolutionary significance of geographic variation (Niewiarowski and Roosenberg, 1993).

The goal of life history theory is to explain variation in life history strategies observed in natural populations (Jones, Ballinger and Porter, 1987). Traditionally, it was assumed that life history strategies represent evolutionary (genetically based) responses to local environmental and demographic pressures, but it is now accepted that variations in life history traits observed among a group of populations arise from both genetic and non-genetic sources (Ballinger, 1983; Jones, Ballinger, and Porter, 1987; Dunham, Miles and Reznick, 1988). Non-genetic sources of variation result from plastic phenotypic responses to local environmental conditions, while genetic differences may result from genetic changes that have accumulated since the populations became separated and diverged from their common ancestor. While interspecific

variation in life histories may be a result of genetic differences (phylogenetic effects), intraspecific variation may be the result of physiological or developmental responses to environmental conditions (e.g. Stearns and Sage, 1980) rather than adaptation to local selective pressures (Stearns, 1980).

Life history traits vary between geographically separated populations, and variation can also occur within a single population over time (Jones, Ballinger and Porter, 1987). Documentation of annual variation in reproductive characteristics within populations of reptiles has helped to elucidate some of the constraints influencing life history tactics at the microevolutionary level (e.g. Schwarzkopf, 1992; Olsson and Shine, 1997). Phenotypic plasticity has been documented for growth rate (Dunham, 1978; Ballinger and Congdon, 1980; Sinervo and Adolph, 1989; 1994), reproductive output (e.g. Ford and Seigel, 1989a; Seigel and Ford, 1991; 1992; Howland, 1992; Rohr, 1997) and age at maturity (e.g. Grant and Dunham, 1990; Mateo and Castanet, 1994; Rohr, 1997).

1.3.2 Approaches to studying life history variation

The comparative method is the keystone of evolutionary ecology (Huey, 1994). Studies of life history variation generally focus on one of two different, but complementary comparisons: variation within versus variation between species (Niewiarowski and Roosenburg, 1993). The comparative approach has been used in numerous interspecific studies, but it is now recognised that these methods are inadequate unless detailed phylogenetic information is incorporated into the analyses (e.g. Felsenstein, 1985).

Phylogeny is one of the strongest influences of life history traits in lizards (Stearns, 1984; Dunham and Miles, 1985b; Dunham, Miles and Reznick, 1988; Miles and Dunham, 1992; Bauwens and Diaz-Uriate, 1997). Many tests of life history theory are based on interspecific comparisons, which often fail to incorporate phylogenetic relationships, especially phylogenetic similarities which may underlie character variation (Miles

and Dunham, 1992). Miles and Dunham (1992) investigated life history variation within the iguanids and found that many important life history traits, including age at first reproduction, adult female size, average clutch size and neonate size, were correlated with phylogeny. Tinkle (1969) first stressed the importance of using the same or closely related species for the study of life history patterns:

> "...in fact, intraspecific comparisons may be more powerful than interspecific ones because of the uncontrolled phylogenetic variable in the latter."

and Tinkle, Wilbur and Tilley (1970) argued that:

> "more detailed intraspecific studies of reproductive parameters are necessary to understand selective processes within species populations and to strengthen hypotheses about the origin of reproductive strategies".

Therefore, it is argued that interspecific comparisons of life history traits provide only limited help in identifying the causes of differences, because often the differences that exist can be explained in terms of phylogenetic and design constraints (e.g. Dunham and Miles, 1985b) and consequently the use of intraspecific comparisons is more powerful (Jones and Ballinger, 1987).

Investigation of intraspecific geographic variation in life history is especially useful in determining the relationship between life history characteristics and environmental conditions (Howland, 1992). Environmental variation may be coupled to life history variation both proximally, through its influence on genotype through ontogeny and ultimately, as a source of selection on life history genotype (Niewiarowski and Roosenburg, 1993). The relative contributions of genetic and environmental sources of phenotypic variation are rarely determined, especially for vertebrates. Distinguishing between the sources of variation brought about by genetic and non-genetic sources is a

rapidly expanding area in herpetology, but much research on a range of species occurring over wide variety of habitats is still required.

Many studies have documented variation in life history traits and attributed these to proximate sources. Such sources include food or energy availability (e.g. Ballinger, 1977; Dunham, 1978), environmental gradients (Ballinger, 1983; Grant and Dunham, 1990; Rohr, 1997), and habitat type (Ferguson, Bohlen and Wooley, 1980; Tinkle and Dunham, 1986). Temperature has also been identified as an important source of variation in lizard life histories (e.g. Huey and Stevenson, 1979; Beuchat and Ellner, 1987; Grant and Dunham, 1990; Sinervo, 1990a; Adolph and Porter, 1993; 1996). Adolph and Porter (1993; 1996) developed models to describe the proximate effects of temperature on lizard life history traits, including fecundity, survival, and size and age at maturation. In their models, the thermal environment affects life history through its influence on daily and seasonal activity time.

The most promising line of investigation used to identify sources of variation in life history characters involves the use of common garden laboratory techniques (e.g. Ferguson and Brockman, 1980; Sinervo and Adolph, 1989; Sinervo, 1990a; Ferguson and Talent, 1993) or reciprocal transplant experiments (Ballinger, 1983; Bernado, 1993; Niewiarowski and Roosenburg, 1993). However, until recently these techniques were rarely applied to lizards and general patterns are only beginning to emerge. In most of these investigations, evidence for interpopulation differences that may reflect genetic differences has been found in addition to strong environmental effects. These techniques will improve the degree to which observed variation in life history traits may be attributed to evolved genetic differences, and the degree of variation that may be attributed to proximate sources acting on the expression of the phenotype.

More recently, manipulations of phenotypes have proved valuable in understanding and testing life history evolution. These manipulations may either be through direct physical manipulation of offspring size (for example "allometric engineering" (e.g. Sinervo 1990b; Sinervo and Huey,

1990; Sinervo and Licht, 1991a; 1991b; Sinervo *et al.*, 1992) or through manipulations of the environment (e.g. Shine and Harlow, 1993; 1996; Ferguson and Talent, 1993; James and Whitford, 1994). In both these approaches, the phenotypes are altered and their "fitness" is tested, either through performance measures or through examination of growth rates. These experimental approaches have the advantage that they can be used to test predictions from life history theory on optimal traits in different environments.

1.3.3 Biases in lizard life history studies

Dunham, Miles and Reznick (1988) suggested that there has been a bias in studies to date, with a few species studied extremely rigorously. Although studies of reproductive ecology in reptiles have proliferated over the last fifteen years, data are sufficient to allow analysis of geographic variation in reproductive characteristics and demography for only a few species with broad geographic distributions (Dunham, 1994).

Studies of life history and demographic variation within and between lizard populations have become increasingly prominent in attempts to refine and test current theories of life history evolution (Ruby and Dunham, 1984). However, several inadequacies exist. There is still a paucity of data for certain taxa (including Scincidae) and our understanding is based on generalisations from a few groups only. Furthermore, variation in life history characteristics of viviparous species remains particularly poorly known. There is also an insufficient number of studies comparing life histories and demography of populations of single species over a wide range of environments (Tinkle and Dunham, 1986; Ruby and Dunham, 1984; Jones and Ballinger, 1987).

Knowledge about the causal mechanisms responsible for variation in life history parameters is poor, and data about intraspecific geographic variation in many families of reptiles (including skinks) are rare (Dunham, Miles and Reznick, 1988). Our knowledge of the ecological and evolutionary significance of life history diversity in lizards is based

primarily on studies of North American iguanomorph lizards (e.g. Sinervo and Adolph, 1989; Sinervo 1990a; 1990b; Ferguson and Talent, 1993; Adolph and Porter, 1993; 1996). However, the high degree of phylogenetic conservatism in life histories of reptiles (Stearns, 1984, Miles and Dunham, 1985b; Dunham, Miles and Reznick, 1988) means that independent data sets are required for species from other lizard taxa that are phylogenetically well separated, in order to fully understand general patterns of covariation among life history traits and relationships between life history characteristics and environmental variables (Forsman and Shine, 1995). Few Australian skinks have been the subject of detailed life history studies (Hutchinson, 1993) and even fewer have been a source for data of geographic or annual variation in life history traits.

1.4 Australian lizards

The Australian lizard fauna is represented by over 500 described species from five families: Agamidae (dragon lizards), Gekkonidae (geckos), Pygopodidae (legless lizards), Scincidae (skinks) and Varanidae (goannas) (Cogger, 1993).

Skinks constitute the largest and most diverse family of lizards in the world (Greer, 1989; Hutchinson, 1993). There are approximately 1000 species, which is about 25% of the total known species of lizards. In Australia and its external territories there are 325 species of skink in 32 genera (as of December, 1990, from Hutchinson, 1993) and these constitute over 50% of all known lizards within Australia (Wilson and Knowles, 1988). New species continue to be described. In Australia and its external territories, all skinks belong to the subfamily Lygosominae (Greer, 1989; Hutchinson, 1993). This lineage accounts for nearly two thirds of all skink species and much of the family's distribution (Greer, 1989). The Australian genera may be further divided into three monophyletic assemblages, informally termed the *Egernia* Group, the *Eugongylus* Group and the *Sphenomorphus* Group (Hutchinson, 1993).

Skinks in Australia display both oviparous and viviparous modes of reproduction. Viviparous species tend to be more common in cooler climates or high altitudes and latitudes (Greer, 1989; Shine, 1985). Live-bearing is thought to have arisen independently on at least 11 occasions in Australian skinks and eight of these taxa have a southern distribution (Shine, 1985). Brood size varies enormously in skinks, ranging from 1 to 53; it is generally variable within a species and is usually correlated with female size (Taylor, 1985; Greer, 1989). Greer (1989) provided a comprehensive table of the available data on Australian skinks, summarising their modes of reproduction, average brood size and their relative clutch masses (Tables 9 and 11 in Greer, 1989). Despite the obvious importance of this family, both in the Australian environment, and in the reptile world generally, there have been few comprehensive studies of annual or geographic variation in life history traits.

1.5 Tasmanian reptiles

The Tasmanian reptile fauna has been described as relatively impoverished (Heatwole, 1976; Hutchinson, Schwaner and Medlock, 1988). Discounting the occasional aquatic visitor, there are only twenty species of reptile described from three families: the Scincidae (16 species), the Agamidae (1 species) and the Elapidae (3 species). The majority of the skinks were, until recently, placed in the genus *Leiolopisma* (Wilson and Knowles, 1988; Ehmann, 1992) or *Pseudemoia* (Cogger, 1992), but it is now recognised that both these genera are polyphyletic, (Greer, 1989; Hutchinson *et al.*, 1990; Hutchinson and Donnellan, 1992).

Prior to the taxonomic work of Hutchinson and co-workers (Hutchinson, Schwaner and Medlock, 1988; Hutchinson, Robertson and Rawlinson, 1989; Hutchinson *et al.*, 1990; Hutchinson and Schwaner, 1991) much confusion surrounded the taxonomy of Tasmanian lizards. Recently a number of the Tasmanian species from the genus *Leiolopisma* were included in a major taxonomic revision by Hutchinson *et al.* (1990). An important result of this study was the formation of four new genera including the largely Tasmanian endemic genus *Niveoscincus*. The

revised classification is as follows: ***Bartella*** (gen. nov.)*: jigurra;* ***Bassiana*** (gen. nov.): *duperryi, platynotum, trilineata;* ***Cautula*** (gen. nov.): *zia;* ***Niveoscincus*** (gen. nov.): *coventryi, greeni, metallicus, microlepidotus, ocellatus, orocryptus, palfreymani, pretiosus;* ***Pseudemoia*** (Fuhn, 1967): *baudini, entrecasteauxii* (Group 1 and Group 2), *rawlinsoni, spenceri* (Table 1.1). More recent revision (Hutchinson and Donnellan, 1992) has further clarified the taxonomy of *Pseudemoia,* and Rounsevell, Brereton and Hutchinson (1996) provided further detail on the species within the genus *Pseudemoia* in Tasmania.

The Tasmanian reptile fauna shows a pattern of adaptive responses to the relatively cool, humid climate. All but one species are heliothermic, actively basking to elevate body temperatures above the generally low air temperature. There is evidence, however, that alpine species also use thigmothermy to extend the time available for activity (Melville and Swain, 1997a). Only one species, *Lerista bougainvillii* is a true thigmotherm and its distribution is limited to the warm open habitats of the eastern Bass Strait islands, only colonising the Tasmanian mainland on its two northeastern promontories (Rounsevell, Brereton and Hutchinson, 1996).

The Tasmanian reptile fauna is dominated by viviparous species. There are three egg-laying species (*Tympanocryptus diemensis, Bassiana duperryi,* and *Lampropholis delicata*) that depend on a warm microclimate for successful incubation of eggs. As a result these species are confined to the warmest parts of the state (Rounsevell, Brereton and Hutchinson, 1996). Interestingly, a fourth species, *Lerista bougainvillii,* gives birth to live young in Tasmania and lays eggs in some mainland Australian populations (Greer, 1989; Qualls and Shine, 1995; Qualls, 1997).

Despite recent changes in the taxonomy of Tasmanian skinks, little recent ecological study has been undertaken on them, or indeed on any other member of Tasmania's herpetofauna. The most important published work on the ecology and biogeography of Tasmanian reptiles remains that of Rawlinson (1974). His account of the present-day

Table 1.1. The current classification of the Tasmanian terrestrial reptile species.

order	sub-order	family	sub-family	genus	species	common name
Squamata	Lacertilia	Agamidae		*Tympanocryptus*[1]	*diemensis*	mountain dragon
		Scincidae	Lygosominae	*Bassiana*[2]	*duperryi*	three lined skink
				Lampropholis[3]	*delicata*	delicate grass skink
				Lerista	*bougainvillii*	Bougainvilles skink
				Niveoscincus[3]	*metallicus*	metallic skink
				Niveoscincus[3]	*ocellatus*	Tasmanian spotted skink
				Niveoscincus[3]	*pretiosus*	Tasmanian tree skink
				Niveoscincus[3]	*greeni*	northern snow skink
				Niveoscincus[3]	*microlepidotus*	southern snow skink
				Niveoscincus[3]	*orocryptus*	mountain skink
				Niveoscincus[4]	*palfreymani*	Pedra Branca skink
				Pseudemoia[3]	*entrecasteauxii*	southern grass skink
				Pseudemoia[3]	*pagenstecheri*	tussock skink
				Pseudemoia[3]	*rawlinsoni*	glossy grass skink
				Egernia	*whitii*	Whites skink
				Tiliqua	*nigrolutea*	blotched blue-tongue
				Cyclodomorphus[5]	*casuarinae*	she-oak skink
	Ophidia	Elapidae	Elapinae	*Austrelaps*	*superbus*	copperhead snake
				Drysdalia	*coronoides*	white-lipped or whip snake
				Notechis	*ater*	tiger snake

1 - previously *Amphibolurus*
2 - previously *Leiolopisma trilineatum*
3 - previously *Leiolopisma*
4 - previously *Pseudemoia*
5 - previously *Tiliqua*

distribution of Tasmanian reptiles is based on detailed taxonomic, ecological and distributional studies of the reptiles of southeastern mainland Australia and its offshore islands. Rawlinson (1974) concluded that the present-day distribution of Tasmania's herpetofauna is largely a reflection of past major glacial events, when Tasmania was connected to the mainland by an extensive land bridge, the "Bassian Isthmus".

Faunal interchanges are most likely to have taken place when the climate was much colder than at present, thus limiting the dispersal of many species into Tasmania (Rawlinson, 1974). The last major glacial period commenced in the Late Wisconsin period, reaching a maximum around 20 000-18 000 years before present. At this time sea levels were at their lowest. Sea levels then began to rise and by 5000 years before present the current coastline was formed (Rawlinson, 1974). Presently the climate reflects an interglacial period, with mild temperatures and high sea levels (Heatwole and Taylor, 1987). The present day distribution and speciation of members of the genus *Niveoscincus* are currently the focus of a detailed study (Melville, pers. comm.). This study will undoubtedly add much to our understanding of the importance of past glacial activity in shaping the present day distribution of species within *Niveoscincus*.

The main zoogeographical subregions into which Australia is divided correspond closely to the major climatic divisions. Rawlinson (1974) identified three subregions: the temperate Bassian, tropical Torresian and arid Eyrean. Heatwole and Taylor (1987) suggested that the Bassian region is more accurately divided into three separate areas: (1) the southwest subregion in southwestern Australia; (2) the Kosciuskan subregion on the southeastern mainland; and (3) the Tasmanian subregion. The climate within the Tasmanian subregion is characterised by lower temperatures than mainland Australia with a winter maximum rainfall (Heatwole and Taylor, 1987). Zoogeographic subregions are a convenient way of classifying reptile faunas if one recognises their oversimplified nature (Heatwole and Taylor, 1987).

There are only a limited number of published works on Tasmanian skinks (but see below for work on *Niveoscincus* species) and few of these

are ecological. Rounsevell, Brereton and Hutchinson (1996) briefly describe the reptiles of the north-east of the State, in which a new key for the genus *Pseudemoia* is included. They also add that "basic ecological knowledge of many Tasmanian species is incomplete or missing".

1.5.1 The genus *Niveoscincus*

Niveoscincus ("snow skink") is the largest genus of reptiles in Tasmania and has a predominantly Tasmanian distribution. Seven of the eight species occur in Tasmania, and of these, six are endemic. The eighth species, *N. coventryi,* is a highland species, occurring from the Blue Mountains in New South Wales to the Grampians in Victoria on mainland Australia only (Cogger, 1992). *Niveoscincus ocellatus,* the focus of the present study, is one of the endemic species. The genus can be further divided into two eco-morphological subgroups. Two species, *N. metallicus* and *N. coventryi,* are large-scaled, strictly terrestrial species while the majority are small-scaled climbers. Five species, *N. greeni, N. microlepidotus, N. orocryptus, N. pretiosus* and *N. palfreymani,* have restricted and/or isolated habitats (Hutchinson, Robertson and Rawlinson, 1989; Hutchinson *et al.,* 1990; Rawlinson, 1975; Rounsevell, Brothers and Holdsworth, 1985) and two, *N. metallicus* and *N. ocellatus,* have a widespread distribution.

The ground-dwelling nature of the two species not endemic to Tasmania (*N. metallicus* and *N. coventryi*) suggests an evolutionary scenario starting with the colonisation of Tasmania by a "metallicus-like" form with the scansorial (climbing) habit common to the endemic forms subsequently evolving within Tasmania along several separate morphological lineages (Hutchinson, Robertson and Rawlinson, 1989). Of the derived species, *N. ocellatus* is the most morphologically distinct and most differentiated genetically (Hutchinson and Schwaner, 1991).

The three exclusively alpine species *N. greeni, N. microlepidotus* and *N. orocryptus* represent another divergence, with these species thought to be very closely related, indicating recent divergence (Hutchinson,

Schwaner and Medlock, 1988). These species show the greatest divergence in terms of ecology, morphology and behaviour from the type species (Hutchinson, Schwaner, and Medlock, 1988). Recently, Melville (Melville, unpublished data) has undertaken a detailed analysis of phylogeny within the *Niveoscincus* genus and examined the evolution of the scansorial (climbing) habit within this genus through the use of ecomorphological analyses. Her work will undoubtedly add to our understanding of the present day distribution and speciation within *Niveoscincus*.

Until recently, much of the recent published work on *Niveoscincus* has been restricted to phylogenetic analyses (Hutchinson, Schwaner and Medlock, 1988; Hutchinson, Robertson and Rawlinson, 1989; Hutchinson *et al.*, 1990). However, recently there have been a number of other studies, including those concerned with reproductive cycles (Swain and Jones, 1994; Jones and Swain, 1996; Jones, Wapstra and Swain, 1997), reproductive physiology (Swain and Jones, 1997; Stewart and Thompson, 1994), daily and seasonal activity patterns (Melville and Swain, 1997a), spatial and temporal separation between species (Melville and Swain, 1997b), and feeding ecology (Wapstra and Swain, 1996). This genus provides an excellent group in which to examine reproductive adaptation to cool/cold temperate climates and to examine radiation/speciation because of its relatively recent colonisation of Tasmania and subsequent speciation.

All *Niveoscincus* species are viviparous, a characteristic that is generally regarded as an adaptation for cool climates (Shine and Bull, 1979; Shine, 1983; Qualls, 1997). Recently, *N. metallicus* has been used (Jones and Swain, 1996; Swain and Jones, 1994; 1997) as a model species to examine the evolution of viviparity in cool and unpredictable climates. For example, Swain and Jones (1997) demonstrated the transfer of organic nutrients across the placenta in *N. metallicus*, a mechanism that may allow a more flexible approach to reproduction in unpredictable climates. Further details concerning the reproductive cycles of *Niveoscincus* are provided in Chapter 3.

1.5.2 *Niveoscincus ocellatus*

Niveoscincus ocellatus is a small to medium sized skink with an adult snout vent length of 60-80 mm and weighing 2-10 g (Figure 1.1). This species is viviparous and breeds annually across its range, producing a clutch of 1-8 young in January/February each year. It is generally believed that copulation occurs in autumn with fertilisation in spring (Rawlinson, 1974). *Niveoscincus ocellatus* is widely distributed within Tasmania, occupying rocky outcrops from sea level to about 1000 m (Rawlinson, 1974; Wapstra and Swain, 1996). Climatically, this encompasses both cool temperate and cold temperate zones (Rawlinson, 1974). The Tasmanian climate is characterised by both seasonal and diel uncertainty and cold weather; snow at high altitude may be experienced in any month of the year. Despite its distribution across a wide range of habitats and climates this species has received relatively little attention from researchers. *Niveoscincus ocellatus* is primarily a rock dweller and is believed to represent one lineage of scansoriality (climbing habit) within the genus (Hutchinson and Schwaner, 1991).

This species is active from the beginning of spring (September) until late autumn (May/June) with occasional non-feeding emergence in winter in warmer cool temperate areas (Wapstra and Swain, 1996). In cold temperate regions, the activity is restricted to approximately October to April, with an unbroken winter torpor (pers. obs.). *Niveoscincus ocellatus* is particularly common on the east coast of Tasmania, where it is the dominant reptile species in rocky outcrops, particularly dolerite scree slopes and granite areas. Its distribution is more restricted in the cooler areas of the state, particularly in the south and west. Its altitudinal distribution is also extensive. It is common at sea level on the warm east coast, and there are mountain populations throughout the Central Plateau where the species occurs sympatrically with the closely related alpine specialist, the northern snow skink, *N. greeni* (Rawlinson, 1975; pers. obs.). Figure 1.2 shows the known distribution of *N. ocellatus* within Tasmania based on records of the Tasmanian Parks and Wildlife Service (TASPAWS).

Figure 1.1. Typical female *Niveoscincus ocellatus* from the Central Plateau of Tasmania. This individual is a gravid female with a snout vent length of 72 mm and weighing ~ 8 grams.

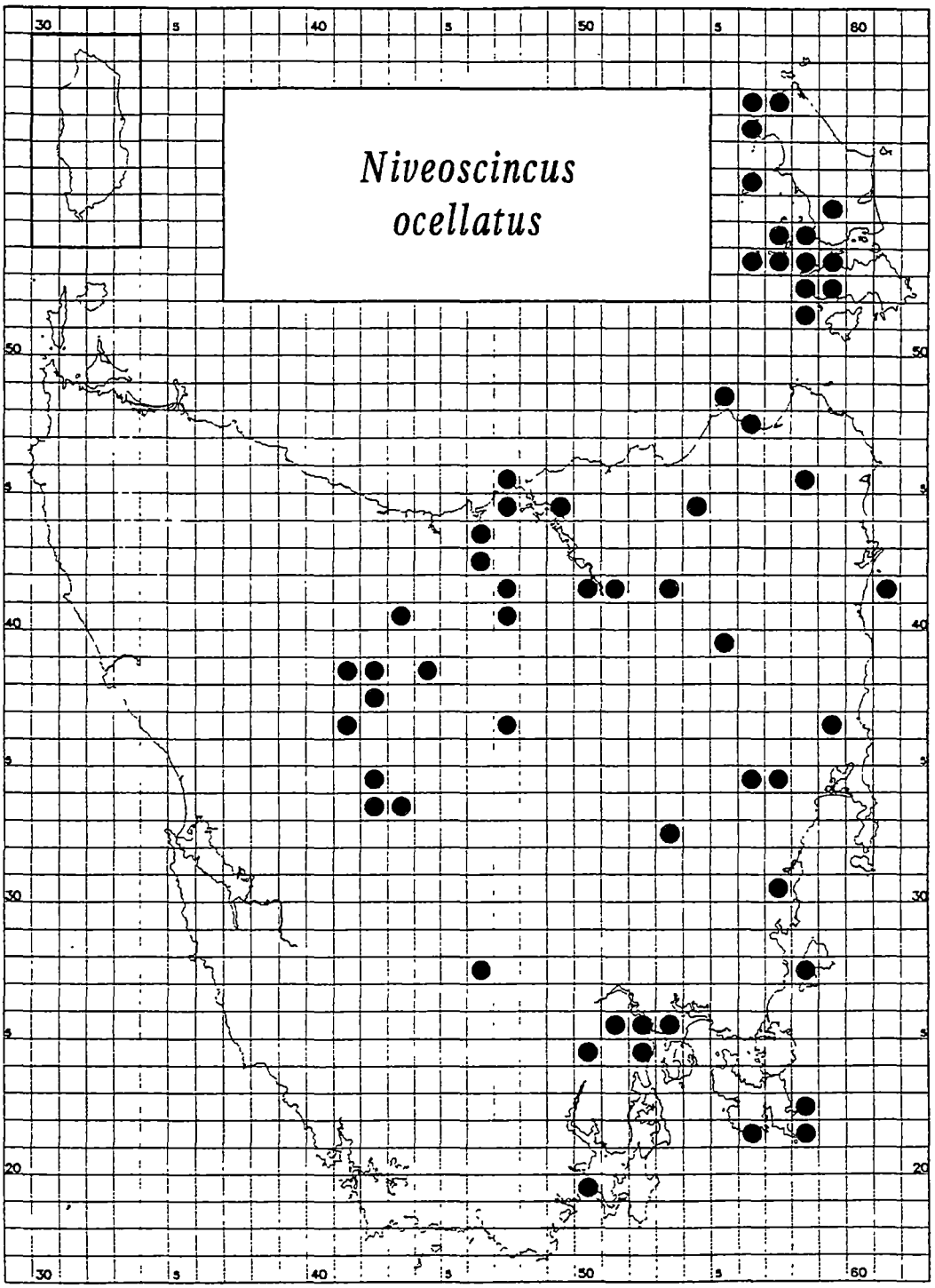


Figure 1.2. Distribution of *Niveoscincus ocellatus* (map courtesy of David Peters (Department of Environment and Land Management) from TASPAWS records).

Recently, there have been some more detailed studies of *N. ocellatus*, including Wapstra and Swain (1996) and Wapstra (1993) in which the feeding ecology and basic reproductive details were described from one population on the east coast of Tasmania. Jones, Wapstra and Swain (1997) described the male and female reproductive cycle with an emphasis on the asynchronous gonadal cycles and plasma concentrations of male and female sex steroids.

Due to its widespread distribution within Tasmania, *N. ocellatus* provides an ideal study organism for examining adaptations to cold environments and geographic variation in life history patterns. Initial surveys of populations from the climatic extremes revealed that this species displays interesting variations in life history patterns that have not been previously documented. This thesis presents data on these variations from two wild populations at the climatic extremes of the species' distribution (see Chapter 2 for study site details), as well as some experimental approaches designed to contribute to an understanding of the variation in life history patterns.

Chapter 2

Study sites

2.1 Location of study sites

Niveoscincus ocellatus occurs over a wide geographical range within Tasmania. However, for this comparative study two major study sites were chosen. The first site (the Orford study site) was located near the township of Orford on the East Coast of Tasmania and is typical of much of the coastal cool temperate region where *N. ocellatus* is particularly common. The second study site (the Central Plateau study site) was located adjacent to Lake Augusta, near the township of Liawenee and is typical of much of the Central Plateau region within Central Tasmania. This site is within the cold temperate region (Rawlinson, 1974) and may be described as subalpine. These two localities represent two ecologically different environments at the climatic extremes of the species' distribution. Figure 2.1 shows the location of the study sites within Tasmania.

2.2 Description of study sites

2.2.1 Orford study site

The Orford study site is located 1 km south of the township of Orford adjacent to the Tasman Highway (42°34'S, 147°52'E) and has been little disturbed by recent human activity although there is evidence of a bushfire 5-10 years ago. It lies 50-75 m above sea level and forms part of a dolerite scree slope, providing an abundance of shelter and basking sites. Figure 2.2 illustrates the typical habitat at the Orford study site. The vegetation is dominated by dry, open woodland species including the white peppermint (*Eucalyptus pulchella*), black peppermint (*Eucalyptus*

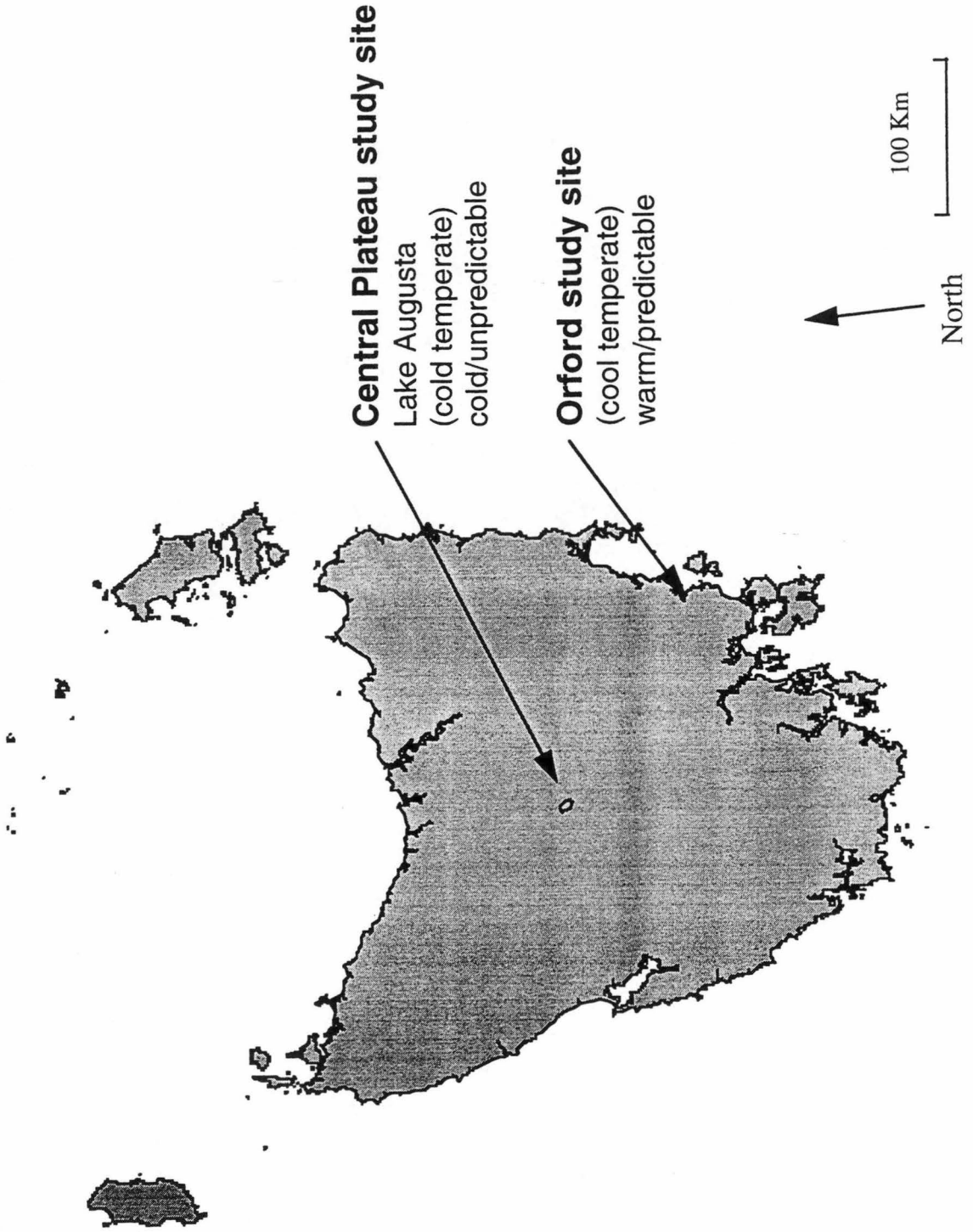


Figure 2.1. Location of study sites within Tasmania. These study sites are separated by a distance of ~ 200 km. Although there are populations of *Niveoscincus ocellatus* throughout this range, the study populations are discontinuous.

amygdalina), silver wattle (*Acacia dealbata*), she-oak (*Allocasuarina stricta*) and Oyster Bay pine (*Callitris rhomboida*). The most common plant species at ground level include various grasses such as kangaroo grass (*Themeda triandra*) and sagg (*Lomandra longifolia*), as well as native cranberry (*Astroloma humifusum*) and the pink mountain berry (*Cyathodes* sp.).

This study site was also used to examine the feeding ecology of *N. ocellatus* (Wapstra, 1993; Wapstra and Swain, 1996) and reproductive and steroid cycles in *N. ocellatus* (Jones, Wapstra, and Swain, 1997).

This site is particularly suitable for examination of life history characteristics because it carries a large population of *N. ocellatus*, likely to be able to withstand regular sampling without seriously affecting numbers. Furthermore, it constitutes a simple homogeneous environment of uniform substrate and vegetation so that sampling may be conducted over a relatively large area. The site supports an abundant reptile fauna, with many species co-existing: *Niveoscincus metallicus*, *N. ocellatus*, *Pseudemoia entrecasteauxii*, *Bassiana duperryi*, *Egernia whitii* and *Tympanocryptus diemensis* were seen in large numbers. *Tiliqua nigrolutea*, *Niveoscincus pretiosus* and *Notechis ater* were also seen during the study period.

2.2.2 Central Plateau study site

The Central Plateau study site is located adjacent to Lake Augusta on the Central Plateau, approximately 15 km from Liawenee (41°51'S, 146°34'E). The man-made lake wall provides an abundance of shelter and basking sites (Figure 2.3), although population densities of *N. ocellatus* were very similar in nearby natural rock screes. The site lies approximately 1200 m above sea level.

The vegetation is dominated by subalpine grass species with some shrubs. The site, in comparison to the Orford study site, is more open with little ground cover. The man-made dam wall is composed of small

to medium sized dolerite boulders. The majority of work was conducted on the dam wall; however, the adjacent rock screes along the lake edge provided further individuals. In addition, in the 1996/1997 season some animals were obtained from a quarry site 300-400 metres away. Although this site was composed of a modified habitat, it supported a large, stable population of *N. ocellatus* in a climate very different to that of the Orford study site. Few other reptile species were present in large numbers, although *N. metallicus* was present. This species was restricted to nearby areas with more ground cover. *Niveoscincus ocellatus* was the dominant species in the more open scree areas. In addition, the snake species *Notechis ater* and *Drysdalia coronoides* were seen. Other areas in the nearby vicinity including natural rock screes within a few hundred metres, also supported similarly large stable populations of *N. ocellatus* from which collections were made.

This site is particularly suitable for examination of life history characteristics because it carries a large population of *N. ocellatus* close to its climatic and altitudinal limit which is likely to be able to withstand regular sampling without seriously affecting population size. *Niveoscincus ocellatus* occurs commonly throughout the Central Plateau, to elevations of 1300 m above sea level, and at these limits occurs sympatrically with *N. greeni* (Rawlinson, 1975; pers. obs.). At these sites the true alpine specialist species, *N. greeni*, makes reproductive concessions in response to the cold conditions and occurs at higher altitudes than *N. ocellatus* is able to occupy.

a.

b.

Figure 2.2. Typical habitat at the Orford study site

a.

b.

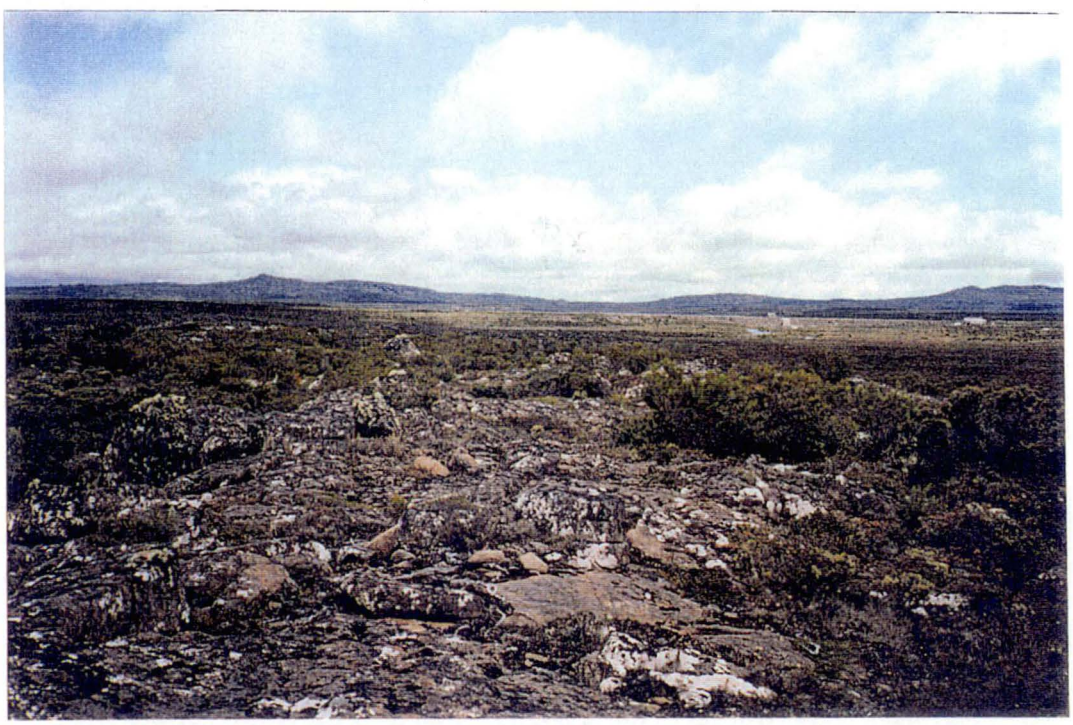


Figure 2.3. Habitat at the Central Plateau study site; a) man made dam at Lake Augusta, both sides were used for collection of lizards, b) nearby habitat that was occupied by *Niveoscincus ocellatus*.

2.3 Comparison of climatic conditions

The two study sites differ markedly in climatic conditions. The Orford study site is described as cool temperate, while the Central Plateau study site may be described as cold temperate (Rawlinson, 1974). Climatic data were obtained from records from the Bureau of Meteorology. Table 2.1 displays the position and elevation of the weather station nearest to the study sites. No data for sunshine are available from the Orford weather station; the nearest comparable site on the East Coast of Tasmania is at Bicheno.

Table 2.1. Details of Bureau of Meteorology weather stations used for climate data during the period of the study

station	site number	latitude	longitude	elevation	dates
Orford	092027	42°33'06" S	147°52'39" E	15 metres	Jan '92- June '97
Liawenee	096065	41°53'54" S	146°40'06" E	1060 metres	Jan '92- June '97
Bicheno	092003	41°52'27" S	148°18'06" E	10 metres	Jan '92- June '97

2.3.1 Temperature profiles

The two study sites differ markedly in temperature. Throughout the entire year Orford is considerably warmer than the Central Plateau, in terms of both maximum and minimum monthly air temperatures (Figures 2.4 and 2.5). The temperature at any time of day throughout the year is considerably higher at Orford than at the Central Plateau although the differences are less during summer (Figure 2.6). Differences in temperature during the day between the study sites are not as large during the warm summer months, although at night the differences remain large. This is reflected in the temperature differences at 9 am which are larger than at 3 pm.

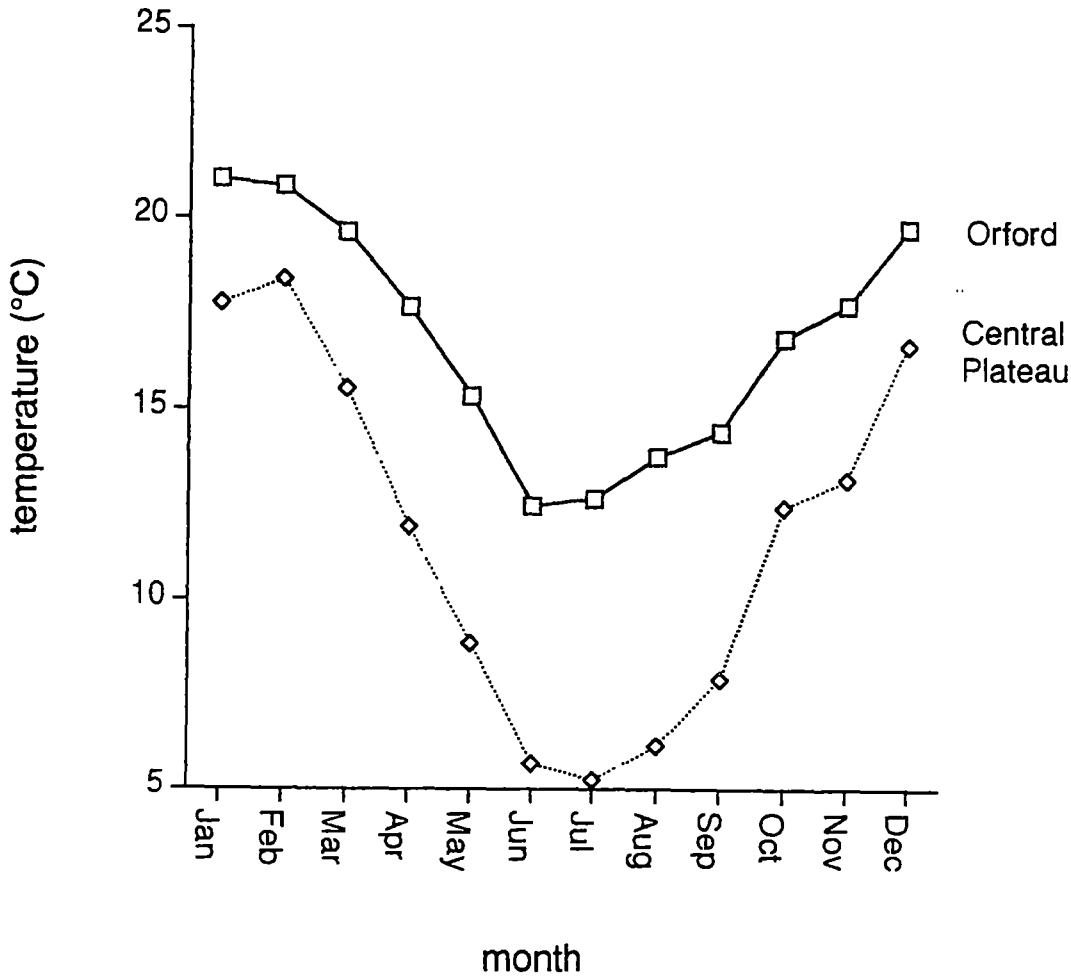


Figure 2.4. Mean monthly maximum air temperatures (°C) for 1992-1997 at the Central Plateau and Orford study sites.

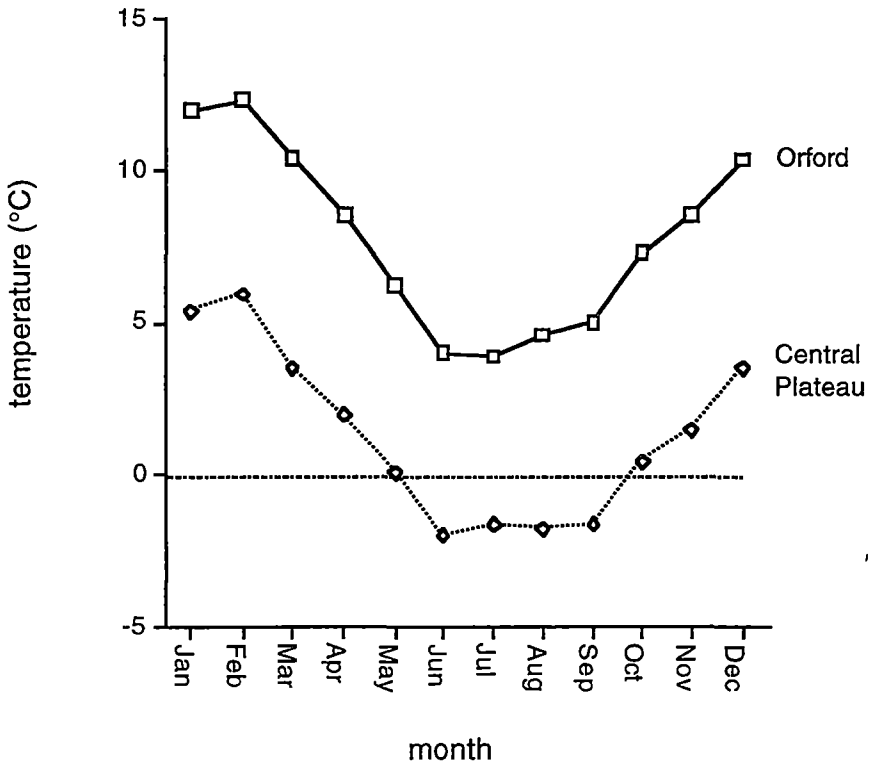


Figure 2.5. Mean monthly minimum air temperatures (°C) for 1992-1997 at the Central Plateau and Orford study sites.

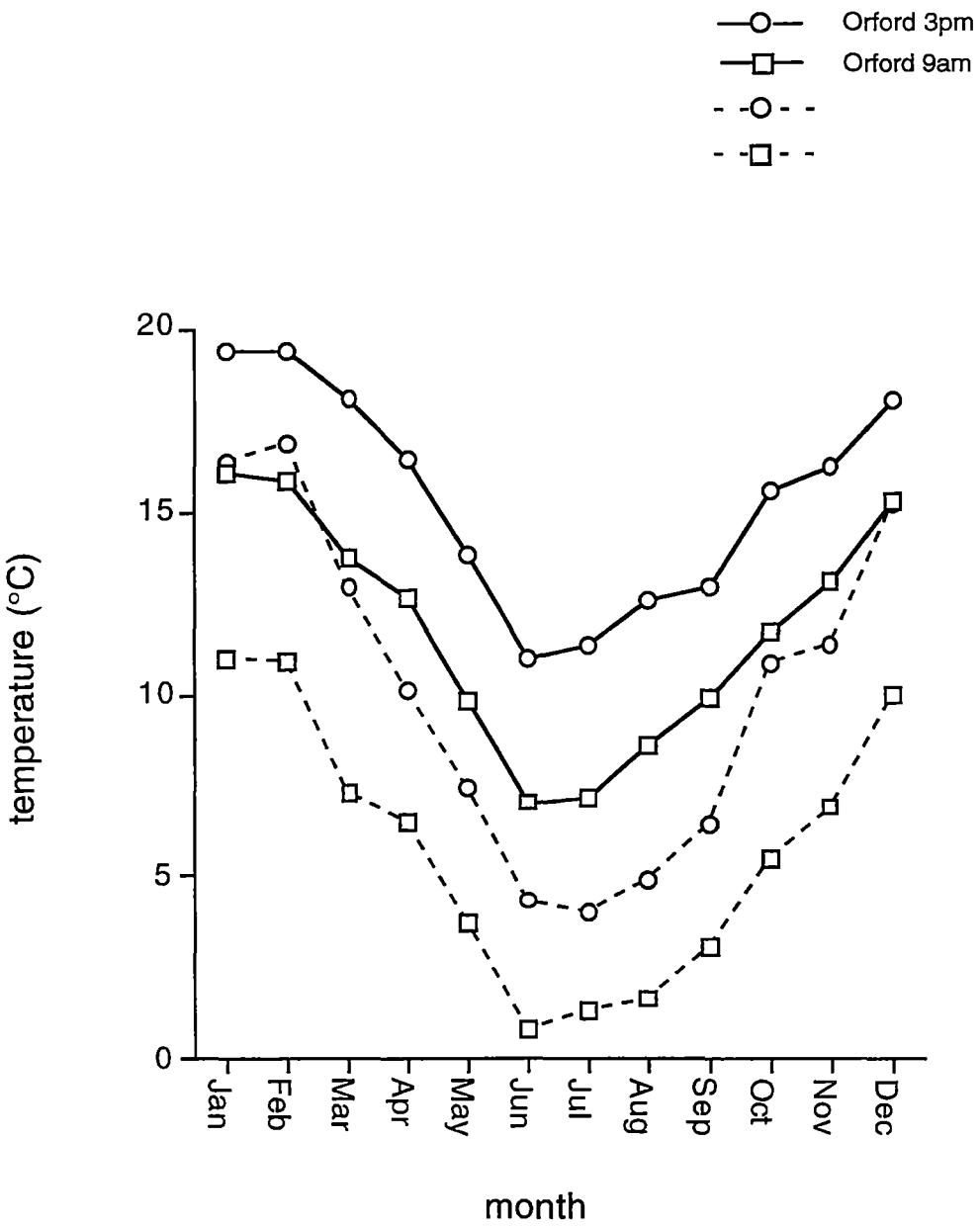


Figure 2.6. Mean monthly air temperatures (°C) at 9 am and 3 pm at the Central Plateau and Orford study sites for 1992-1997.

2.3.2 Precipitation levels and cloud cover

There are marked differences in the levels of precipitation at the two study sites (Table 2.2). The Central Plateau study site receives approximately twice the amount of rain that falls at Orford. Predictably, there is also a large difference in the number of days on which rain falls at the sites (150 days at Orford and 212 days at the Central Plateau). To further highlight the differences between the sites, the Central Plateau receives snow on an average of 63 days each year, while Orford receives snow only very occasionally. Snow may be expected in any month at the Central Plateau (pers. obs.).

Table 2.2. Summary of annual precipitation levels and the number of days on which snow fell at the study sites.

year	Rain (mm/year)		Rain days		Snow days	
	Orford	Central Plateau	Orford	Central Plateau	Orford	Central Plateau
1992	572.5	1085.2	158	197	0	61
1993	634.2	1072.4	143	222	0	42
1994	375.8	1079.2	121	192	2	67
1995	804.2	940.6	147	214	1	73
1996	773.8	1426.9	183	235	0	73
average	**632.1**	**1120.9**	**150**	**212**	**0.6**	**63**

Although the Central Plateau receives considerably more precipitation, and has more days of precipitation, there is little difference in the number of cloudy and clear days at the two sites (Table 2.3).

Similarly, there is little difference in the amount of bright daily sunshine (Figure 2.7) received at both sites, although the Central Plateau receives more sun during the warmer months of November to February. A similar pattern is exhibited by cloud cover (Figure 2.8), with few differences during winter months, but a greater proportion of cloud cover at Orford during the summer months. Although cloud cover and

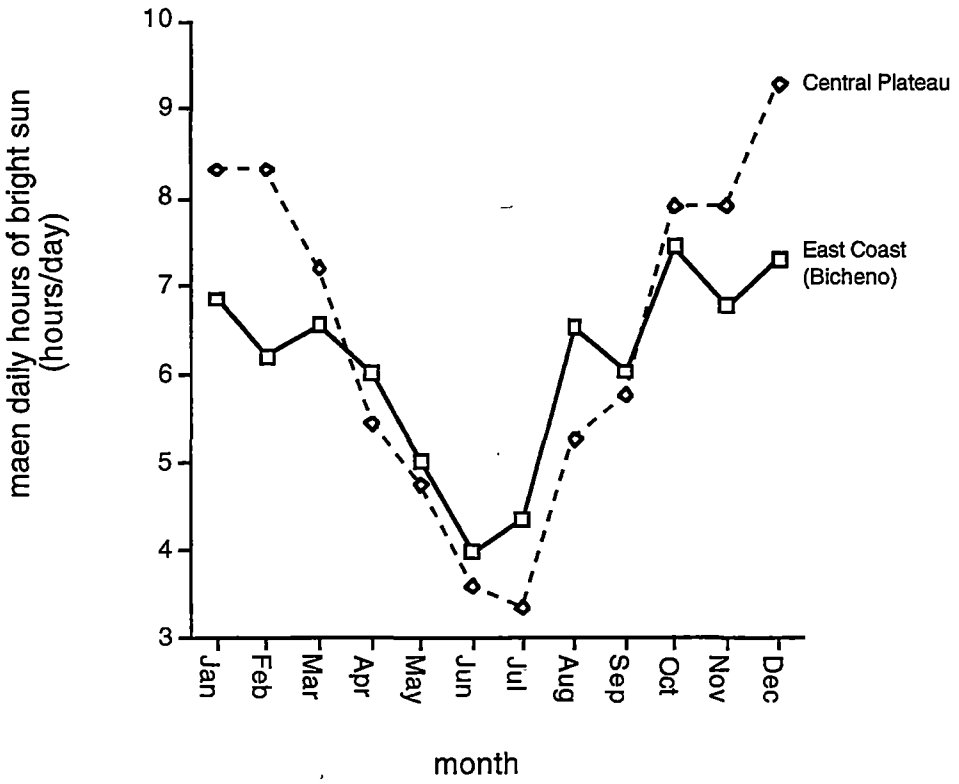


Figure 2.7. Mean daily hours of bright sun (hours/day) at Bicheno and the Central Plateau. Bicheno is the closest weather station to the Orford study site that provides records of daily sun

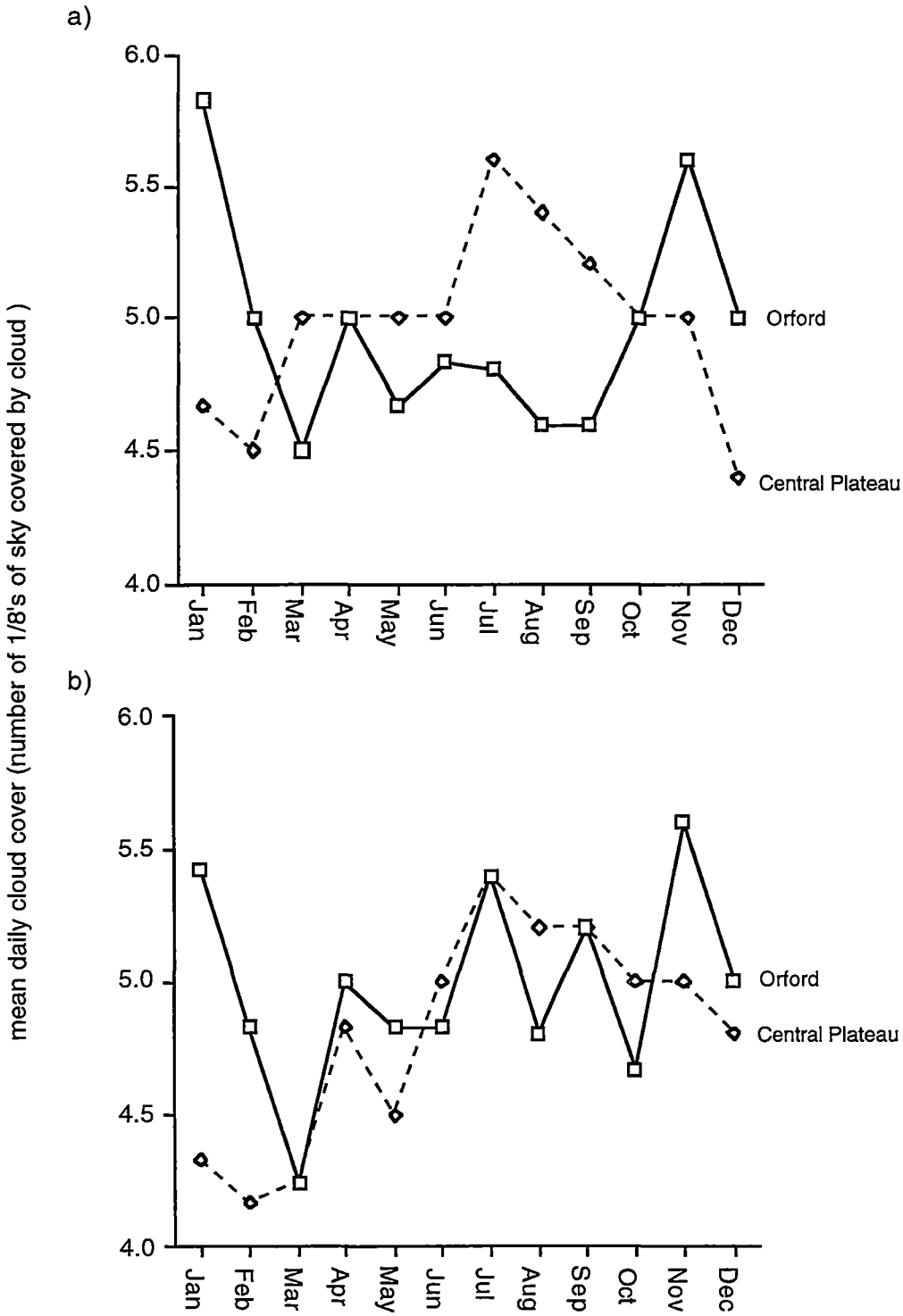


Figure 2.8. Mean monthly cloud cover (proportion) at the Central Plateau and Orford study sites (1992-1997) at 9 am (a) and 3 pm (b).

sunshine are important determinants of lizard activity, lizard activity at the Orford study site often continues under cloud cover because of the relatively high ambient air temperature (Figures 2.4, 2.6), while cloud cover and precipitation at the Central Plateau often limit lizard activity because of the low ambient air temperature (pers. obs.).

Table 2.3. Summary of clear and cloudy days at the two study sites.

	cloudy days		clear days	
year	Orford	Central Plateau	Orford	Central Plateau
1992	213	158	43	62
1993	196	147	64	78
1994	148	148	63	86
1995	142	175	67	68
1996	178	186	67	57
average	**175**	**163**	**61**	**70**

2.4 Lizard capture and handling

Several methods of lizard capture were used at the two study sites. The main method used in 1994 and 1995 involved noosing the animals using nylon nooses and long (180-200 cm) fibreglass fishing rods. This method was particularly effective in the rock screes where lizards could not be recovered from beneath rocks. At the Orford study site, this method was supplemented with hand capture after turning of rocks or logs. This was especially effective if a lizard was first sighted before it took cover. Lizards at the Central Plateau typically retreated deep within the rock screes and could not be caught by turning rocks and other cover.

In 1996 and 1997 lizards were also caught by "fishing" (Hodson, 1991). In this method a mealworm is tied to a piece of cotton and hung in front of the lizard. When the lizard caught the mealworm, they were lifted from

their basking surface and placed into a bucket or caught by hand. This method proved very successful in attracting lizards from beneath cover, and catch rates were extremely high, particularly in warm weather when the lizards were actively foraging.

Lizards were generally held in cloth or plastic bags in the field before they were transferred to terraria for transport to the laboratory. Animals required for later experimentation were held in the laboratory under standard conditions (14L: 10D light cycles) with 10 hours of access to basking lamps until they were transferred to their experimental conditions. In the laboratory lizards were housed in plastic terraria (30 cm x 20 cm x 10 cm). Each container was provided with cover (either plastic or bark) and basking surfaces. Basking surfaces (terracotta pots) were placed 10 cm below light globes (25 W) which were on timers independent of overhead lighting. The basking surface temperature was typically 25-35 °C and ambient temperature in the rest of the container ~12-18 °C. These conditions allowed the lizards to thermoregulate throughout the period that the basking lights were on. The overhead lighting (14L: 10D) was supplied by fluorescent lighting, ultraviolet tubes (Grow-Lux) and three large phosphorus globes (150 W each) for bright illumination (20 000 lux).

Adult lizards were fed three times weekly with mealworms dusted with vitamin powder, and fresh fruit was provided for variety. Water was available *ad libitum*. Feeding of juveniles is described in detail in the relevant chapters.

Animals for which life history data were required were killed by placing them in a freezer in the manner described by Cogger (1992). Further details of experimental conditions and animal housing are presented in the relevant sections.

Chapter 3

Reproductive cycles in *Niveoscincus ocellatus*

3.1 Introduction

Reproduction in cool climate reptiles is strongly constrained by environmental conditions, perhaps more so than in any other extant vertebrate group. The timing of the reproductive cycle is an important aspect of a species' reproductive strategy (Guillette and Casas-Andreu, 1987; Guillette and Méndez-de la Cruz, 1993). Temperate zone reptiles exhibit distinct seasonal patterns of reproductive activity (Fitch, 1970; Duvall, Guillette and Jones, 1982; Licht, 1984; Méndez-de la Cruz *et al.*, 1988); seasonal fluctuations in the environment result in some periods being more favourable than others for development of eggs and/or initiation of growth of young (Saint Girons, 1985; Heatwole and Taylor, 1987). The success of a species may well depend on the type of reproductive cycle it has and the environmental cues to which it responds in adjusting that cycle (Heatwole and Taylor, 1987).

In temperate zone lizards the most favourable time of year for reproduction is spring/early summer when food and climatic conditions allow a period of juvenile growth prior to winter. Although temperate zone reptiles produce offspring exclusively in the summer season (James and Shine, 1985; Shine; 1985) a diversity of reproductive patterns exists (Méndez-de la Cruz *et al.*, 1988). The most common pattern in oviparous species is that in which males and females exhibit spring gonadal development, courtship, mating, and oviposition (Fitch, 1970; Méndez-de la Cruz *et al.*, 1988); however, many reptiles in cold temperate climates exhibit viviparity, the evolution of which has been linked to living in cold climates (Shine and Bull, 1979; Shine, 1983) or uncertain/unpredictable climates (Licht, 1984; Stewart, 1989).

Temperate viviparous species may show a variety of patterns including predominantly autumn reproductive activity in which pregnancy proceeds over winter, or predominantly spring reproductive activity in which pregnancy occurs over summer. Furthermore, the reproductive cycles of males and females may be asynchronous (e.g. Guillette, 1983; Guillette and Casas-Andreu, 1980; 1987; Méndez-de la Cruz, 1988; Ramirez Pinilla, 1991; Guillette and Mendez-de la Cruz, 1993). Temperate zone skink species similarly display a variety of reproductive patterns (Hutchinson, 1993). Taylor (1985) described four major patterns of reproductive cycle in Australian skinks using a combination of male and female cycles: type I cycles are characterised by spring spermatogenesis and mating with ovulation in spring; type II cycles are characterised by autumn spermatogenesis and mating, with spring ovulation; type III cycles include species with winter to spring spermatogenesis, spring mating and ovulation; and type IV cycles are characterised by year round spermatogenesis and mating, with the ovaries active all year with a peak in spring. Heatwole and Taylor (1987) extended the description of reproductive cycles to include eleven types among Australian reptiles and provide a summary of the variation in reproductive cycles among temperate and tropical species of skink.

The prevalence of asynchronous reproductive activity is still poorly known because relatively few viviparous species have been studied to date (Guillette and Méndez-de la Cruz, 1993). Reproductive asynchrony has been reported in a number of Australian skink genera including *Hemiergis* (Smyth and Smith, 1968), *Pseudemoia* (Hutchinson and Donellan, 1992) and *Niveoscincus* (Swain, 1972; Rawlinson, 1974; 1975; Swain and Jones, 1994; Jones and Swain, 1996; Jones, Wapstra and Swain, 1997). Reproductive asynchrony has been associated with the evolution of a viviparous reproductive mode in some squamate genera (Guillette, 1982).

Previous studies examining variation in reproductive cycles of geographically widespread species have noted a strong phylogenetic conservatism in basic reproductive strategy (Ballinger, 1983; Guillette and Casas-Andreu, 1987; but see Cree and Guillette, 1995). Additional studies are needed to examine the degree of reproductive flexibility species

exhibit in different climatic conditions (Guillette and Casas-Andreu, 1987).

Timing of reproduction so that young begin life under conditions most favourable for their survival and growth has obvious selective advantages, and has been shown to vary latitudinally and with patterns of seasonal change (Heatwole and Pianka, 1993). Reproductive cycles in temperate zone lizards are correlated with environmental variables such as photoperiod, rainfall, temperature, and food availability (e.g. Duvall, Guillette and Jones, 1982; Moore, Whittier and Crews, 1984; Heatwole and Taylor, 1987 (and references therein); Lee *et al.*, 1989; Patterson, 1990; Flemming, 1993a). Temperature is generally considered the most important factor controlling and regulating reproduction in temperate zone lizards (Duvall, Guillette and Jones, 1982). This may be by direct action or due to the existence of a temperature threshold to facilitate a photoperiod response (Flemming, 1993a). Geographic variation in the seasonality of reproduction and flexibility in adjusting to local environmental conditions may be an important prerequisite for widespread occurrence of some species (Lee *et al.*, 1989).

Despite the occurrence of skinks over a wide range of climatic and habitat types within Australia there have been only a limited number of studies examining reproductive cycles in small skinks and few of them have involved species with wide geographic or climatic distributions. Prior to the start of this study there was little published information on the details of reproduction in Tasmanian lizards. As a foundation to further endocrine studies of reproduction in the genus *Niveoscincus*, Swain and Jones (1994) and Jones and Swain (1996) investigated the annual reproductive cycle, including changes in plasma concentrations of major steroid hormones in male and female *N. metallicus*. *Niveoscincus* is an ideal genus to examine the ecophysiology of reproduction in cool/cold climate reptiles because it contains species which inhabit a variety of habitats and climates from lowland dry sclerophyll forests to subalpine rock screes; some species have a very restricted range, while others occur over a wide climatic gradient. Furthermore, some alpine species (*N. microlepidotus* and *N. greeni*) are known to exhibit an unusual biennial reproductive cycle in which gestation in at least some females

occurs over winter and may last for up to fourteen months (Rawlinson, 1975; Hutchinson, Robertson and Rawlinson, 1989; Olsson pers. comm.).

A detailed study of the asynchronous male and female gonadal cycles and plasma steroid concentrations in *N. ocellatus* from the Orford study site has recently been published (Jones, Wapstra and Swain, 1997). The present chapter presents data on reproductive and abdominal fat body cycles of *N. ocellatus* from both study sites. Studies of the patterns of energy storage are common for species from the Northern Hemisphere, but are rarer for Australian species (Taylor, 1986b). Male gonadal cycle data from the lowland population are re-presented from Jones, Wapstra and Swain (1997) with further data on annual variation in female reproductive cycles from both sites. The data involved were collected and analysed by myself for use in that paper. Data on plasma steroid concentrations from Jones, Wapstra and Swain (1997) are also utilised, but these were provided by my co-authors.

The reproductive cycle of *N. ocellatus* from lowland populations conforms to the pattern described for *N. metallicus* (Swain and Jones, 1994; Jones and Swain, 1996; Jones, Wapstra and Swain, 1997; pers obs). The reproductive cycle in *N. metallicus* is characterised by autumn spermatogenesis and mating, with ovulation occurring in early spring, the females having carried sperm over the winter (Swain, 1972; Stanny, 1982; Swain and Jones, 1994; Jones and Swain, 1996). Rawlinson (1974), in a discussion of the biogeography of Tasmanian reptiles, provided brief notes on the reproductive cycle of *N. ocellatus* and it was suggested that copulation occurs in autumn but no supportive data were provided. In this chapter the reproductive and lipid cycles in *N. ocellatus* from Orford and the Central Plateau are described. Variation in the timing of reproductive events annually within sites and between sites is documented.

3.2 Methods

3.2.1 Lizard collection

Lizards were collected from the Central Plateau and Orford study sites at regular intervals throughout the study period. Many of the lizards used to obtain reproductive data were also used in various other experiments (some of which are described elsewhere in this thesis). No data were taken from individuals which were held in captivity for prolonged periods of time (more than approximately two weeks). Lizards were caught using the methods described in Chapter 2. I aimed to collect samples on a monthly basis, but some months are represented by larger samples, either because lizards were available in greater numbers or because these times corresponded to collections for other purposes. Samples were taken at more frequent intervals in October and November to identify the time of ovulation. Collection of females was also concentrated in January (Orford) and February (Central Plateau) as these periods corresponded to parturition in these populations. These females were maintained in terraria in the laboratory under standard heating and lighting (Chapter 2) until they gave birth to accurately establish parturition dates (see Chapter 4 for more details on female and offspring characteristics). Difficulty in obtaining monthly samples from the Central Plateau study site in cooler months resulted in low sample sizes in some months. Although lizards may be collected during winter months at Orford by disturbance of wintering cover or when they emerge briefly on warm days (Wapstra and Swain, 1996), complete inactivity under deep cover in rock screes at the Central Plateau prohibited winter collection from this population.

3.2.2 Lizard autopsy

Lizards were held in plastic or cloth bags in the field and transferred to terraria for transport to the laboratory. Lizards were held in terraria if required for other experiments or immediately killed by placing them in plastic bags at -20 °C (Cogger, 1992). Blood was taken from some lizards for hormone assays, and these lizards were killed by decapitation (Jones,

Wapstra and Swain, 1997). Blood was centrifuged, and the plasma stored frozen.

Subsequently, lizards that were killed by freezing were thawed and measured. Snout vent length (SVL), total length and the position of any tail break was measured to the nearest millimetre. Externally, the presence or absence of copulatory marks in females, caused by the jaws of males on the anterior ventral torso was noted. In *N. ocellatus*, mating marks shaped like inverted "U"s, are clearly visible against the off-white background ventral colour. No marks are present on females during the summer months. The colour of the copulatory marks was used as a guide to the timing of matings. Very fresh marks (days old) have a bruised blue appearance while older marks (one to several weeks old) fade, but are clearly visible as a sharp black outline. Older marks that are two to three months old (e.g. overwintered females) appear grey and diffuse. Marks fade during gestation and disappear altogether by December/January after females moult.

In males at autopsy the dimensions of each testis were measured (maximum length and width to the nearest 0.05 mm). Mean testis volume for each animal was calculated by the formula for the volume of an ellipsoid, $V = 4/3\pi a^2 b$, where a = half the shortest diameter and b = half the longest diameter. The degree of convolution and colour of the epididymides were noted.

In females at autopsy, the number and size (± 0.1 mm) of enlarged vitellogenic follicles or, in post-ovulatory lizards, the number of ovulated ova or embryos together with the number of corpora lutea were noted for each side of the reproductive tract. Embryos were staged according to Dufaure and Hubert (1961). Details of clutch size and size of offspring are presented in Chapter 4. In both sexes the abdominal fat bodies of all sexually mature lizards were removed and their wet weight recorded (± 0.1 mg).

After autopsy lizards were placed in frozen storage in the Department of Zoology, University of Tasmania.

3.2.3Hormone assays

All details of testosterone, progesterone and estradiol assay procedures are found in Jones, Wapstra and Swain (1997).

3.2.4 Statistical analyses

As appropriate to the data set, Analyses of Variance (ANOVA) was carried out using the program *SYSTAT* for the Macintosh version 5.2 (Wilkinson *et al.*, 1992). For testis volume and plasma testosterone, the untransformed data satisfied assumptions of normality and homoscedasticity. Plasma progesterone and estradiol values were log-transformed to meet these criteria. The significance level was taken as $P = 0.05$. Where significant differences were identified, Fisher's Least Significant Difference (LSD) test was used as the *post-hoc* test.

3.3 Results

3.3.1 The female reproductive cycle

Niveoscincus ocellatus has a Type II reproductive cycle *sensu* Taylor (1985), characterised by a primary autumn mating and spring ovulation. In the Orford population ovulation occurs in late September/early October (Figure 3.2) and gestation lasts until January. Most females in the Orford population have ovulated by October 1 and in the Central Plateau population by November 1. There is some evidence of earlier ovulation in both populations. In the 1996/1997 season, ovulation in some lizards from Orford was observed as early as September 7, and at the Central Plateau ovulation began in all years in mid to late October. Similarly parturition dates vary between sites and years. At Orford, parturition began in early January (January 1) in 1994/1995 and 1996/1997 while in 1995/1996 it began later (January 16). Parturition in the Orford population occurred over a one month period, finishing February 2 in 1995, February 26 in 1996 and February 5 in 1997. Parturition in the Central Plateau population occurred later; January 20 - February 14 in 1995, 31 January -

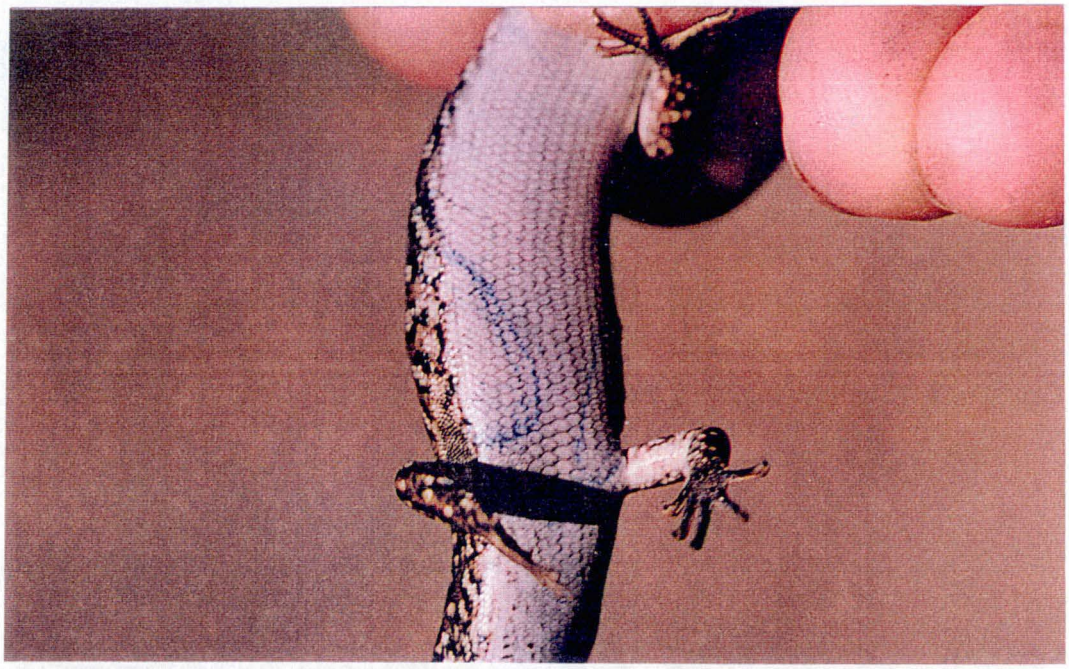
15 February in 1996 and 5 - 18 February in 1997. The summer of 1994/1995 was a wet cold year at the Orford study site, which prevented normal lizard activity and was associated with delayed parturition.

Immediately after parturition the developing follicles were small (1-2 mm). By March, they had enlarged to 2-2.5 mm and become opaque as vitellogenesis began. Copulation first occurred in late March/early April. During copulation, the male grasps the female in his jaws, usually seizing the skin over the shoulders or with the upper jaw on the dorsal surface and the lower jaw on the ventral surface leaving characteristic U-shaped lines termed "mating marks" by Pengilley (1972, cited by Hutchinson, 1993). Mating marks first appeared in late March/early April and by late April all mature females had mating marks. They were clearly visible as "fresh" marks during this time (Figure 3.1 a). By July/August marks were still clearly visible, but appeared grey and diffuse and were therefore scored as old (Figure 3.1 b).

As first discussed in Jones, Wapstra and Swain (1997) there is evidence of a second mating period in late winter/early spring. After winter, mating marks from autumn (Figure 3.1 a) have faded to appear grey/black, rather than having the blue appearance of fresh marks. During spring, some females have fresh marks (blue) superimposed over old (grey/black) autumn mating marks. Mating marks persist through the early stages of gestation but fade in November/December after females shed their skin. Histological examination of oviducts in females in late autumn/early winter revealed masses of sperm in the oviducal lumina and sperm entering gland-like crypts, lined with ciliated cells, in the infundibular region of the oviducts. Histological examination of females with fresh mating marks in spring revealed masses of apparently fresh sperm in the lumina of the oviducts (see also Jones, Wapstra and Swain, 1997).

Vitellogenesis continues through autumn and winter, with follicles continuing to enlarge and becoming yellow and reaching 4.2-5.9 mm in length by July. Follicles continue to enlarge to approximately 10 mm at ovulation in late September/October. Gestation lasts for 12-13 weeks and

a.



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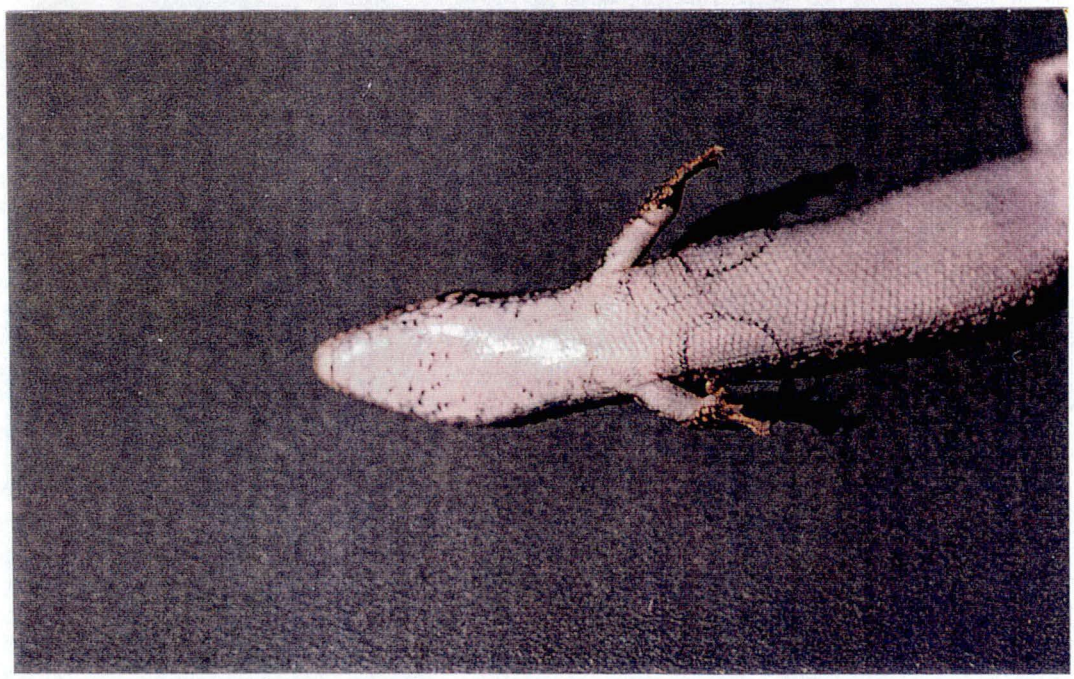

Figure 3.1. Copulation marks on a female *Niveoscincus ocellatus* a. immediately after the autumn mating (note the blue appearance of the marks) and b. following winter the grey/black colour of the marks classifies them as old marks.

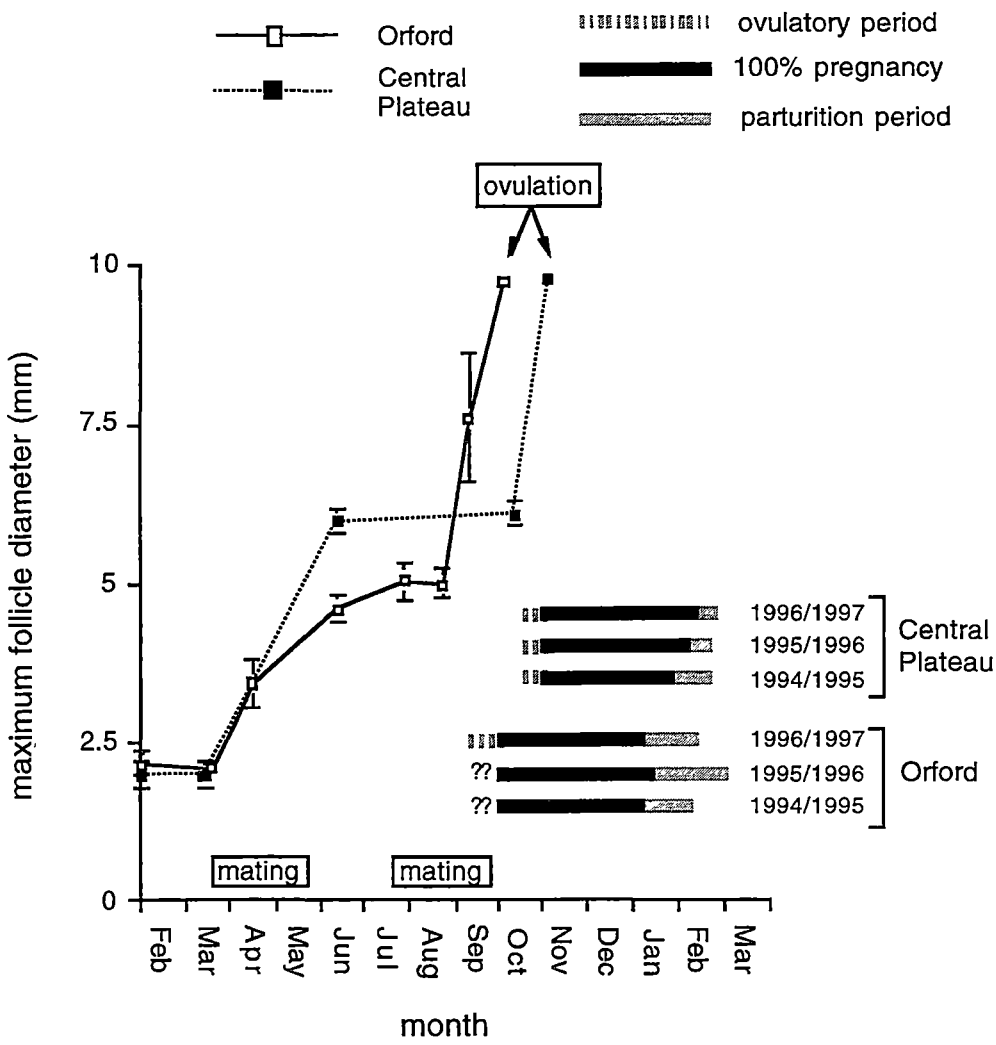

Figure 3.2. Annual female reproductive cycle of *Niveoscincus ocellatus* from Orford and the Central Plateau. Follicle size is mean longest diameter of the largest follicle ± standard error. Gestation periods are indicated for all 3 years of the study (1994-1995; 1995-1996;1996-1997) and the period of parturition for each population indicated. For years where there were sufficient data, the period in which ovulation took place is also indicated.

birth occurs in early to late January. Corpora lutea were present throughout gestation and appeared as ovoid white bodies. The number of corpora lutea usually matched the number of ovulated eggs in the ipsilateral oviduct, but occasionally one egg had swapped to the contralateral oviduct. Data on clutch size are presented as part of an enlarged data set in Chapter 4; however, it is worth noting here that there is little atresia of vitellogenic follicles in the Orford population (0.37 %; Jones, Wapstra and Swain, 1997). Data on vitellogenic follicles are not adequate for the calculation of rates of atresia from the Central Plateau population with confidence, although it appears they are similarly low.

For comparison with hormonal data, four phases of gestation, grouped according to stage of embryonic development were recognised. Early gestation (primitive streak - stage 28) occurred during October. Mid pregnancy (embryos 30-32) occurs during November. Late stage pregnancy is divided into two phases: embryos staged as 36-40, with the most development completed; and late stage (embryos stage 40+, with embryos fully developed and viable at the time of autopsy of the mother). Female hormone data are from the Orford study site and are taken from Jones, Wapstra and Swain (1997) and are presented by reproductive stage: post-partum; vitellogenesis; preovulation; gestation with embryos stage 0-30; stage 31-35; stage 36-40; stage 40+. Low sample sizes at stage 40+ allowed examination of progesterone only.

Figure 3.3 presents the concentrations of estradiol in female plasma. Concentrations differed significantly between reproductive phases (ANOVA: $F_{5,26} = 13.126$, $P < 0.0005$). Estradiol concentrations were significantly lower during late pregnancy (mean ± SE = 158 ± 8 pg/ml) than for any other stage. Concentrations were significantly higher following parturition and during vitellogenesis (means ± SE = 781 ± 124 and 956 ± 214 pg/ml respectively). Preovulatory females had the highest concentrations of estradiol (mean 1241 ± 175 pg/ml) although these were not significantly different from those of postpartum and vitellogenic samples.

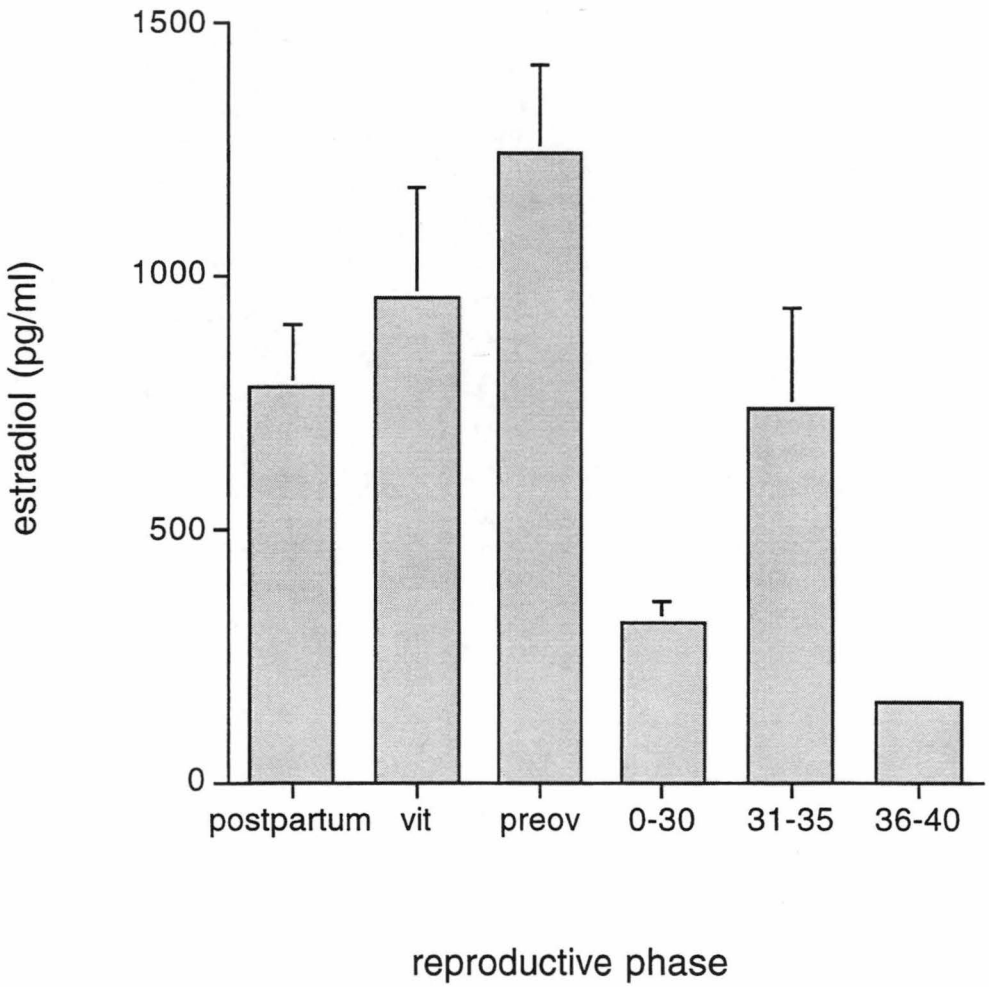

Figure 3.3. Plasma estradiol concentrations (mean ± standard error) in female *Niveoscincus ocellatus* from the Orford study site during the reproductive cycle: previt, postpartum females with vitellogenic follicles; vit, vitellogenic follicles present; preov, females sampled immediately prior to ovulation; 0-30, 31-35, 36-40 represent samples from pregnant females grouped by embryonic stage. Sample sizes are indicated above the error bars (taken from Jones, Wapstra and Swain, 1997).

Plasma progesterone concentrations differed significantly during the reproductive cycle (Figure 3.4; ANOVA: $F_{6,40} = 11.390$, $P < 0.0005$). They were significantly lower in postpartum females (mean ± SE = 0.7 ± 0.1 ng/ml) than at any other time. Concentrations remained low through vitellogenesis and the preovulatory period (mean ± SE = 1.8 ± 0.3 and 2.1 ± 0.3 ng/ml respectively) but rose significantly at the beginning of gestation (mean ± SE = 5.1 ± 0.7 ng/ml). Progesterone plasma concentrations were elevated throughout pregnancy, and were highest during the late stage (embryos stage 36-40) of pregnancy (mean ± SE = 6.5 ± 1.5 ng/ml).

3.3.2 The male reproductive cycle

It was not appropriate to present the male data according to the stages defined for the females because the sexes are out of phase with respect to gonadal development and hormone cycles (Jones, Wapstra and Swain, 1997). As in Swain and Jones (1994), the male data are presented by month of capture, although males collected in October are divided into two groups, pre- and post- ovulation, based on the absence or presence of ovulated follicles in the females collected concurrently (Orford population, Figure 3.5; Central Plateau population, Figure 3.6).

Testis volume at both sites showed a marked seasonal variation (ANOVA: Orford; $F_{10,62} = 7.773$, $P < 0.0001$; Central Plateau; $F_{5,67} = 22.525$, $P < 0.0001$). The testes began to increase in volume in early summer (November-December). Testis volume was significantly higher (mean ± SE = 107 ± 9.9 and 106 ± 14.1 mm^3) during the summer months of January and February than during other months in the Orford population (Figure 3.5). Testes volume showed a similar pattern in the Central Plateau population, although there was a significant peak (mean ± SE = 185 ± 21.1 mm^3) in testes size in February ($P < 0.05$), and showed no difference in December and January ($P > 0.05$). Data from the Central Plateau could not be obtained for examination during autumn, winter

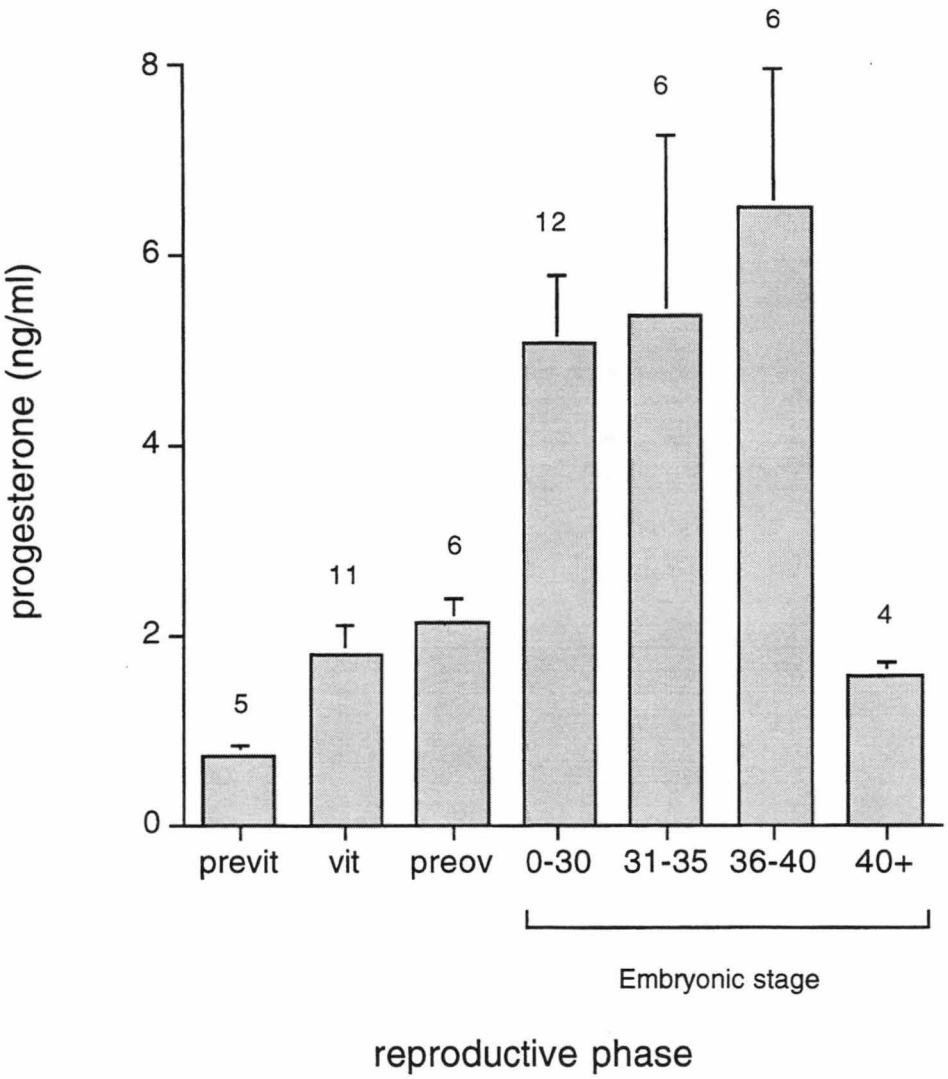

Figure 3.4. Plasma progesterone concentrations (mean ± standard error) in female *Niveoscincus ocellatus* from the Orford study site during the reproductive cycle. Labelling as for Figure 3.3., except that the 40+ sample from pregnant females in which all yolk has been used and embryos are viable has been included (taken from Jones, Wapstra and Swain, 1997).

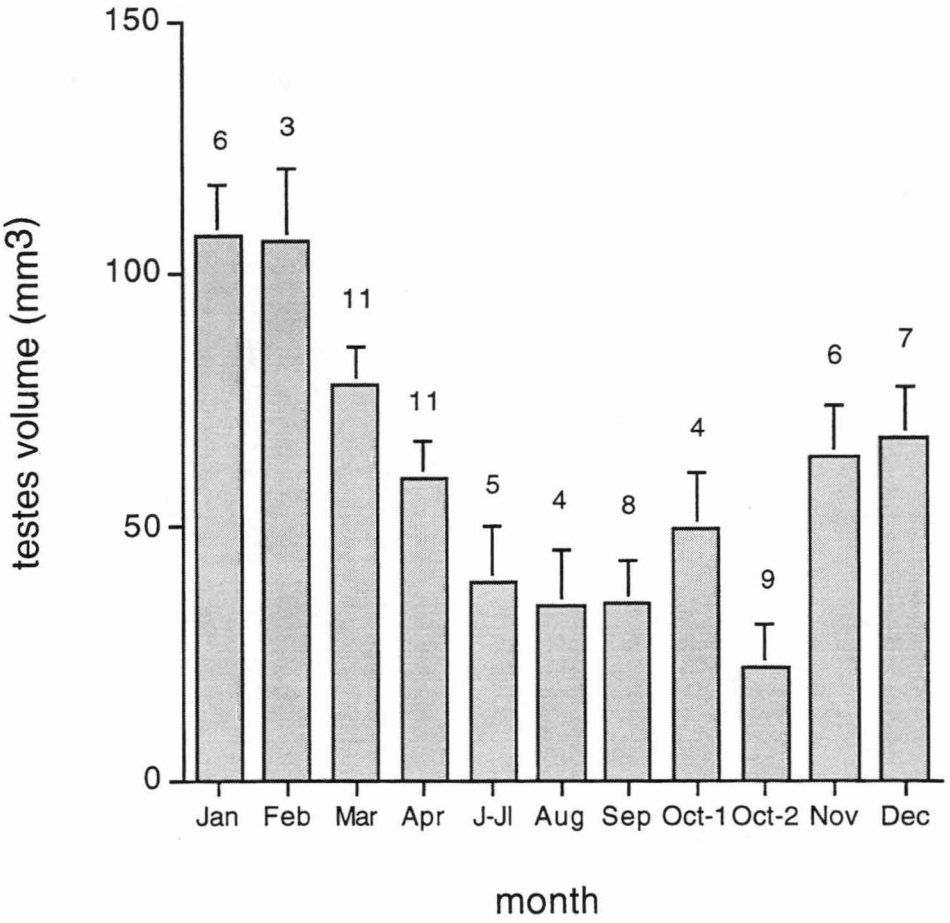

Figure 3.5. Annual variation in testes volume (mean ± standard error) at Orford in male *Niveoscincus ocellatus*. Sample sizes are indicated above error bars. The October sample is divided into two samples; males caught prior to female ovulation (Oct-1) and after female ovulation (Oct-2).

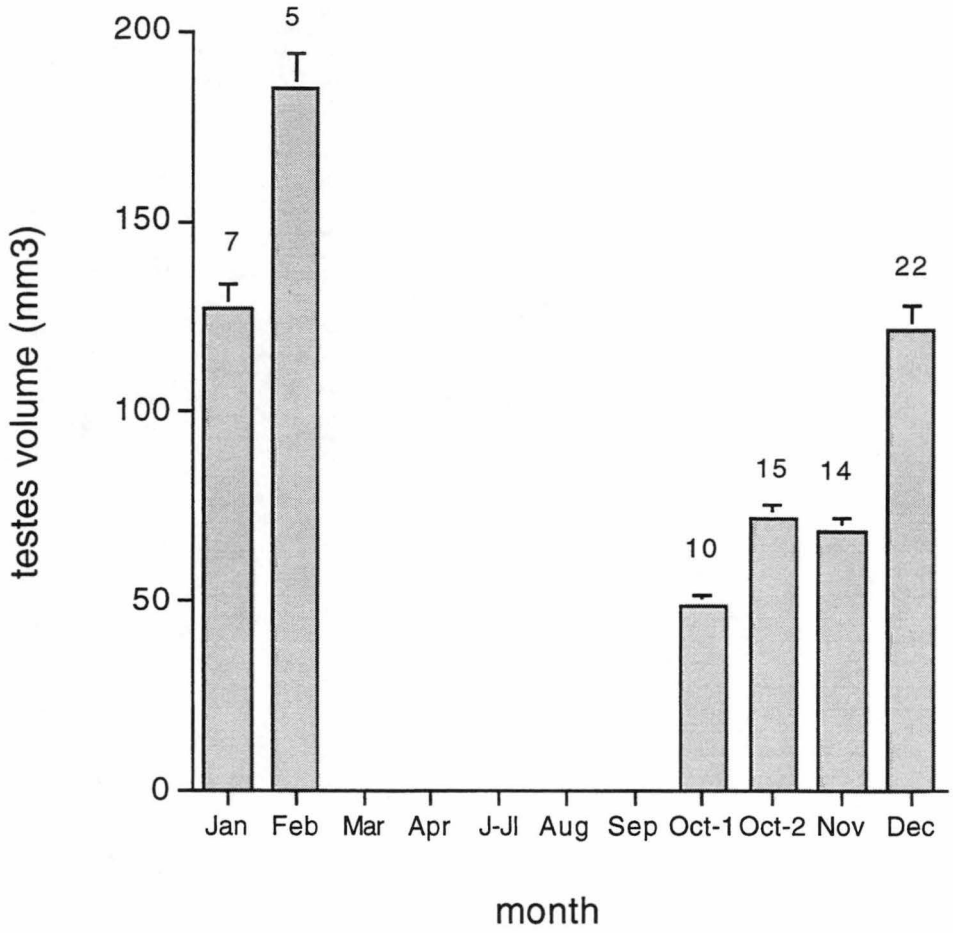

Figure 3.6. Annual variation in testes volume (mean ± standard error) at the Central Plateau in male *Niveoscincus ocellatus*. Sample sizes are indicated above error bars. (No males were sacrificed from March to September as lizards are inactive and inaccessible for most of this period at this site).

and early spring, but testes were smallest in the early October sample. In both populations, testes were smallest in spring (and presumably in winter) and increased through December and January, reaching peak size in February. During the main period of mating (autumn) male hemipenes were readily eversible and males often ejaculated motile sperm. The presence of motile sperm in the ejaculate was confirmed microscopically. During the early spring period of mating males from both populations readily ejaculated and motile sperm were found in the ejaculate .

The epididymides also showed annual changes in appearance. During early summer they were flaccid and clear in appearance. During autumn they became convoluted and white in appearance, indicating the presence of sperm. During this time the sexual segments of the kidneys were well developed. Throughout winter the epididymides remained convoluted and white in colour presumably indicating the presence of stored sperm. The presence of motile sperm in the ejaculate of animals caught around this time support this assumption. In late spring, the epididymides again appeared empty and remained so throughout early summer.

Plasma testosterone concentrations (Figure 3.7) for males from the Orford population are taken from Jones, Wapstra and Swain (1997). There was no correlation between SVL and plasma testosterone concentration. Plasma testosterone concentrations differed significantly during the annual cycle (ANOVA: $F_{10, 53} = 44.048$, $P < 0.0005$). Testosterone concentrations were low through most of the year (means ± SE = 0.7 ± 0.2 and 3.5 ± 0.7 ng/ml), but were high during late summer/early autumn (February mean ± SE = 13.0 ± 1.0 ng/ml; March mean ± SE = 18.6 ± 1.2 ng/ml). These values differed significantly from each other, and both were significantly higher than all other values. Concentrations were lower (mean ± SE = 3.8 ± 1.1 ng/ml) during April when mating occurred. They were significantly lower during winter, with a minor peak in August (mean ± SE = 7.4 ± 1.0 ng/ml) although this value did not differ from the April mean. Concentrations remained low during spring and early summer (September to January) and none of these samples differed

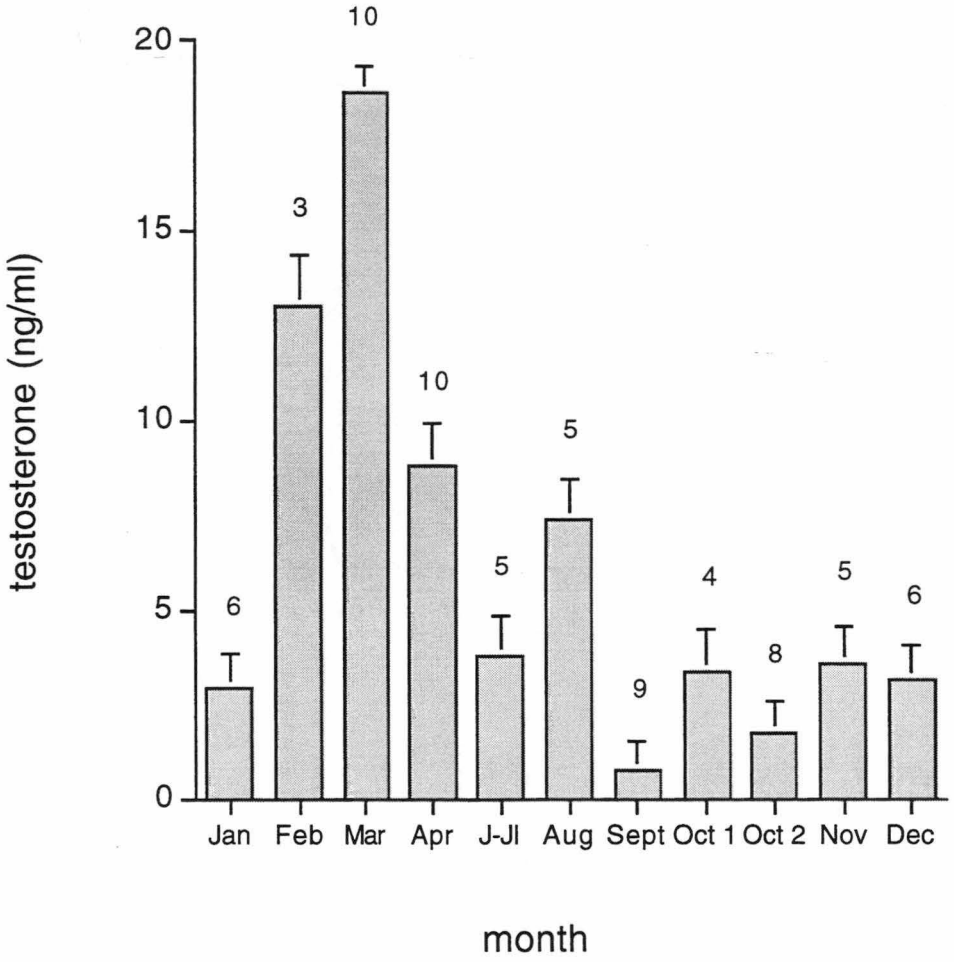

Figure 3.7. Annual variation in plasma testosterone concentration (means ± standard error in male *Niveoscincus ocellatus* from Orford. Sample sizes are indicated above error bars. The October sample is separated into males caught prior to female ovulation (Oct 1) and after female ovulation (Oct 2) (taken from Jones, Wapstra and Swain, 1997).

significantly from each other, although all were lower that the mean for August.

3.3.3 Annual variation in abdominal fat bodies

Abdominal fat bodies were used as a measure of stored lipid in *N. ocellatus*. Data were log-transformed prior to analyses. Figure 3.8 presents the annual variation in abdominal fat body mass for females from both study sites. Both populations show clear patterns of annual variation (ANOVA: Orford; $F_{5,60} = 8.961$, $P < 0.001$: Central Plateau; $F_{5,55} = 11.921$, $P < 0.001$). Sample sizes were low for females from the Central Plateau in some months (N = 2 in January, so these values were excluded from analyses). Sample sizes were also low in November (N = 4) and late October (N = 6), but these values were retained in the above analyses. Combining these two samples into one sample, did not change the significance of the annual variation in fat body masses at this site. In females abdominal fat bodies are small during late vitellogenesis and early gestation but increase in size immediately prior to parturition (January in the Orford females and February in the Central Plateau population). Fat body masses remain high through autumn and the beginning of winter. After winter (during which time vitellogenesis continues) fat bodies are small and remain so during final vitellogenesis and ovulation in spring.

Male *N. ocellatus* show a different pattern of fat deposition to females (Figure 3.9). Males from both populations display significant annual variation in mass of abdominal fat bodies (ANOVA: Orford; $F_{6,47} = 10.503$; $P < 0.001$: Central Plateau; $F_{4,63} = 11.762$, $P < 0.001$). Fat body masses are low in both populations during spring, but increase during summer, when they remain low in females. Fat body mass decreases in males at the Orford site during the period in which gonadal activity is maximum (January-March) and continues to decrease during the major mating period (late March to June).

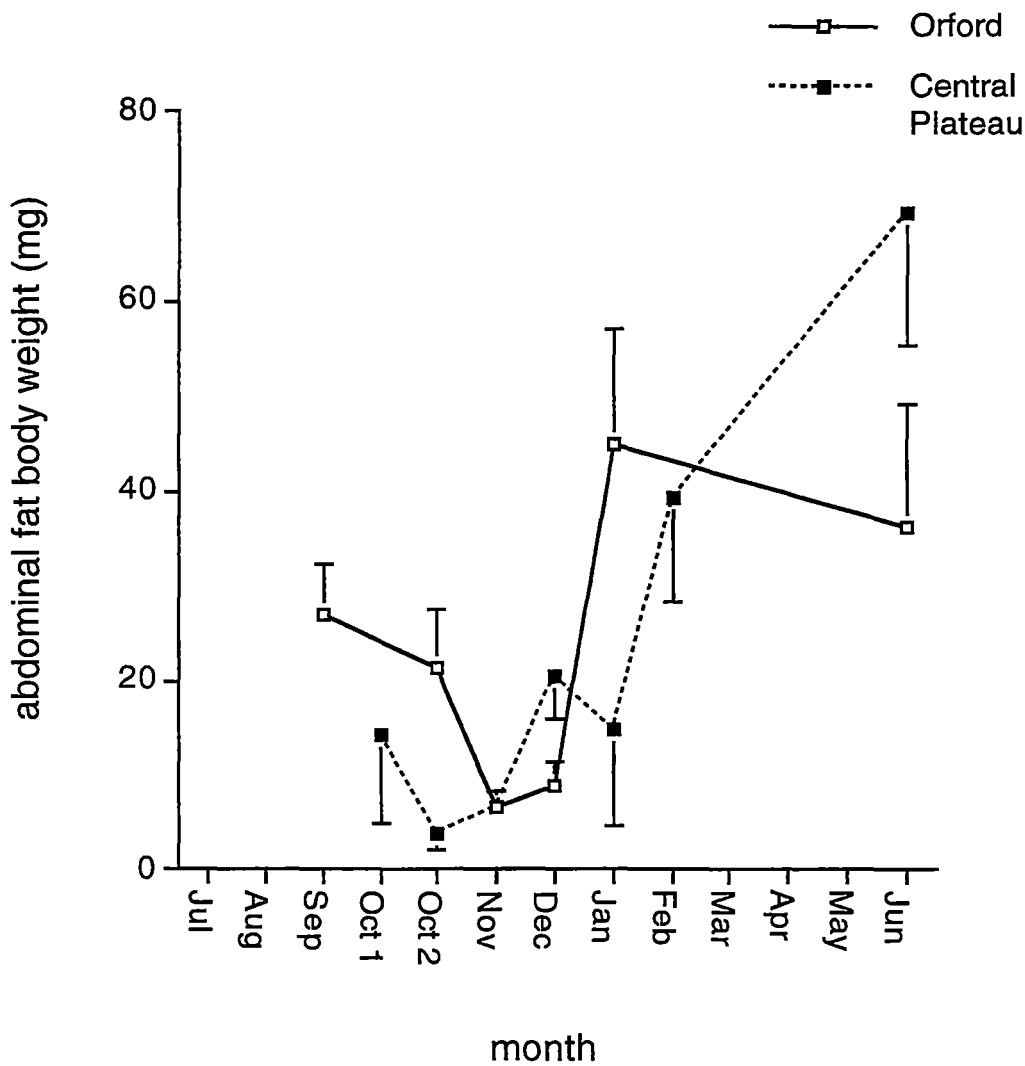

Figure 3.8. Annual variation in abdominal fat body mass (mean ± standard error) for female *Niveoscincus ocellatus* at Orford and the Central Plateau. (Positive standard errors presented for Orford and negative standard errors presented for Central Plateau for clarity).

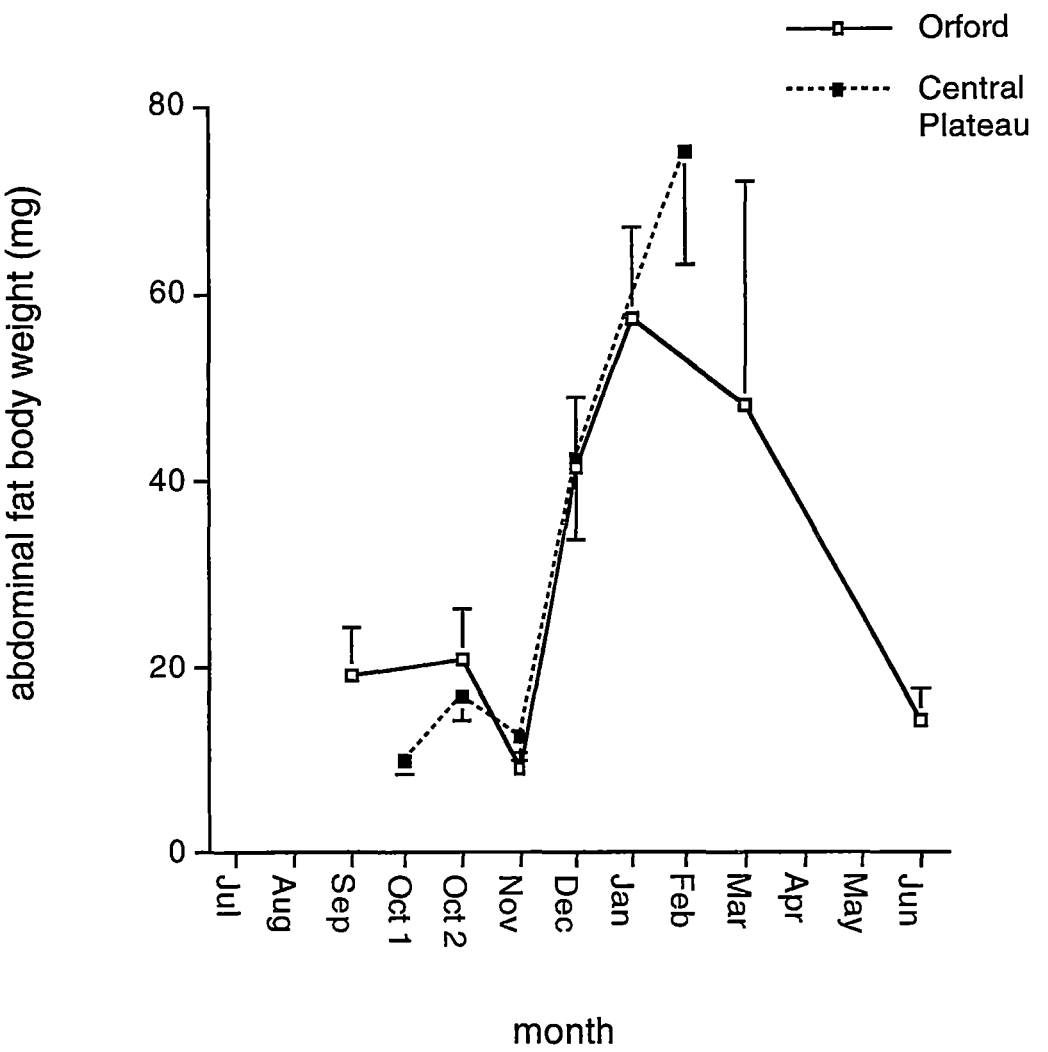

Figure 3.9. Annual variation in abdominal fat body mass (mean ± standard error) for male *Niveoscincus ocellatus* at Orford and the Central Plateau. (Positive standard error presented for Orford and negative standard error presented for the Central Plateau for clarity).

Figures 3.10 and 3.11 present the annual variation in abdominal fat body mass at Orford and the Central Plateau respectively. The difference between sexes in fat deposition is clear, with males increasing the mass of fat bodies during early summer at both sites, while females do not begin to increase in mass until the late stages of gestation (January at Orford and February at the Central Plateau).

3.4 Discussion

Reptiles in temperate zone climates are remarkably consistent in their seasonal timing of reproduction; ovulation typically occurs in late spring, and young are born (or hatch) in late summer (Shine, 1985). No species, except alpine *Niveoscincus* species, are known to depart substantially from this pattern in southeastern Australia and presumably this consistency is enforced by thermal constraints (Shine, 1985). *Niveoscincus ocellatus* fits this pattern of reproduction. Ovulation occurs from September to October in lowland populations and October to early November in highland populations. Gestation continues through spring and the early summer months and young are born in January at the Orford site and late January to mid February at the Central Plateau (Figure 3.2).

3.4.1 Geographic and annual variation in the reproductive cycle

Niveoscincus ocellatus displays only slight differences in the timing of reproductive events from year to year. For example during the 1995/1996 season parturition occurred later than in other years at Orford, while in the same year parturition occurred earlier at the Central Plateau study sites. Differences in the weather at these sites during these years provides an explanation of these differences. The summer of 1995/1996 was characterised by unusually high levels of precipitation and low temperatures at the Orford site, while precipitation was lower than usual at the Central Plateau and temperatures were typical or higher than

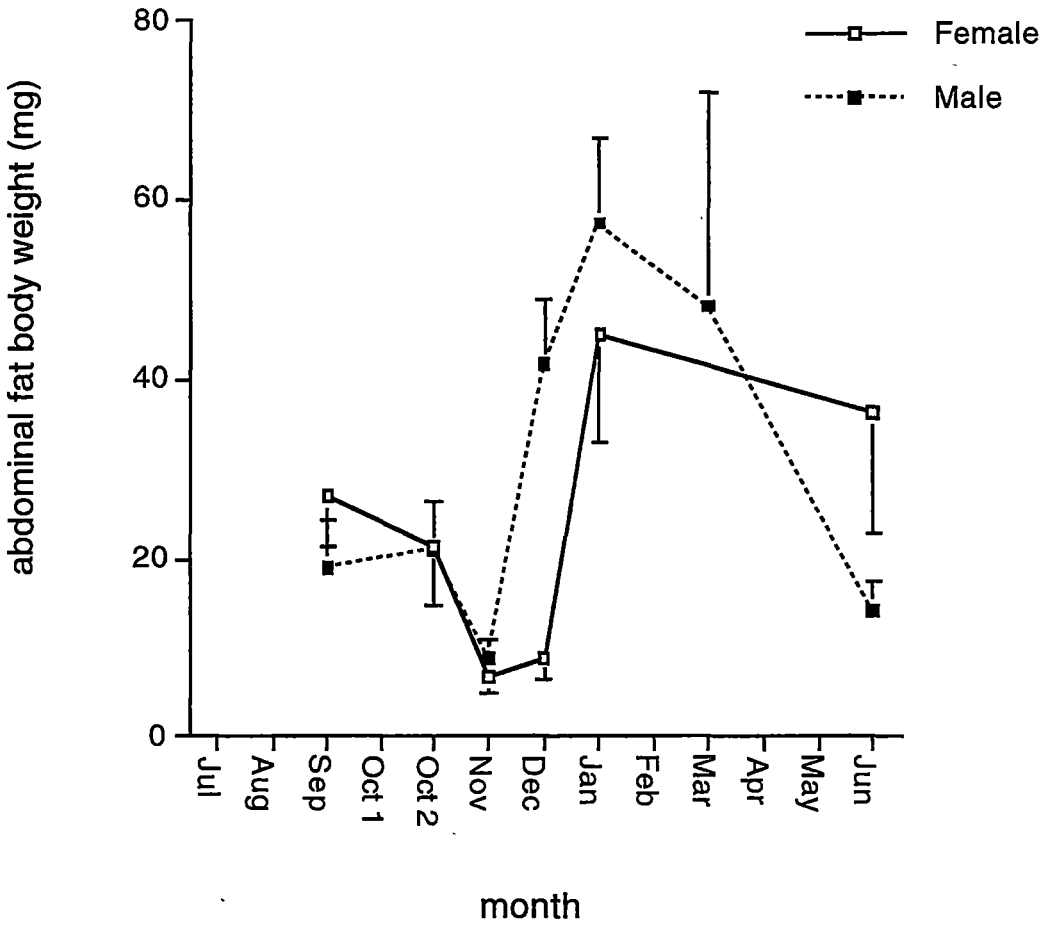

Figure 3.10. Annual variation in abdominal fat body mass (mean ± standard error) for male and female *Niveoscincus ocellatus* at Orford (positive standard errors presented for males and negative standard errors presented for females for clarity).

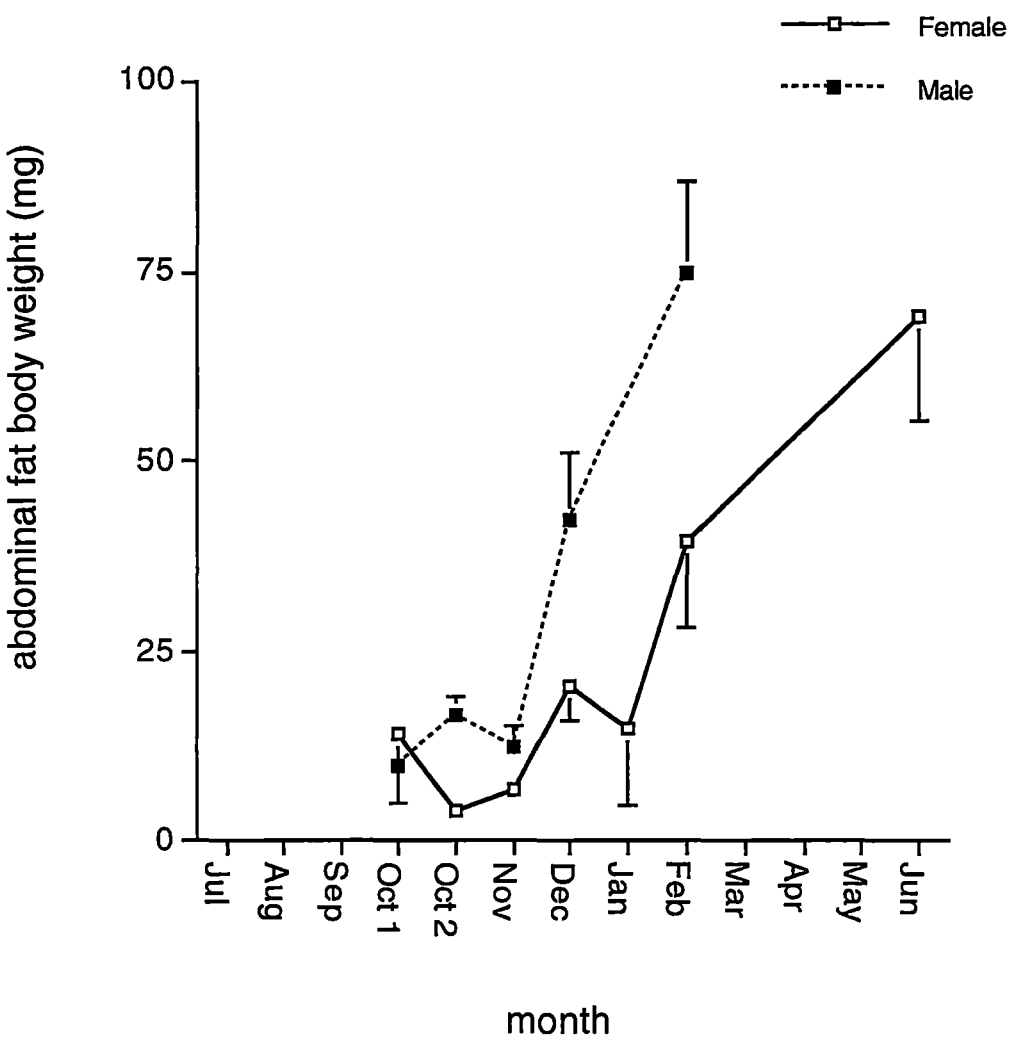

Figure 3.11. Annual variation in abdominal fat body mass (mean ± standard error) for male and female *Niveoscincus ocellatus* at the Central Plateau. (Positive standard errors presented for males and negative standard errors presented for females for clarity).

normal (Table 2.2 in Chapter 2). Small year to year differences in the timing of reproductive events in other species of lizards have also been attributed to differences in temperature and moisture (Goldberg, 1974; 1975; Jones and Ballinger, 1987; James, 1991a).

Although there was annual variation in the timing of reproductive events at each site, there were larger differences in the timing of events between sites. Such differences between geographically and climatically widespread species are not uncommon (e.g. Goldberg, 1974; Beuchat; 1986; Mathies and Andrews, 1995) and are usually related to the different thermal characteristics between sites. Reproductive cycles of temperate zone lizards are correlated with environmental variables such as photoperiod, temperature and food availability (Duvall, Guillette and Jones, 1982; Moore, Whittier and Crews, 1984; James and Shine, 1985; Lee *et al.*, 1989; Patterson, 1990; Flemming, 1993a) and temperature is generally considered to be the most important factor controlling and regulating reproduction in temperate zone lizards.

In *N. ocellatus*, the beginning of gestation (ovulation) is difficult to determine precisely, but in the Orford population it occurs between mid September and early October and births occur from very early January to late January, which gives a gestation length of 90-105 days. Ovulation occurs later at the Central Plateau, but all females have ovulated by November 1. A delay in reproductive events as a result of cool climatic conditions is not always documented. For example, Ballinger and Congdon (1981) and Mathies and Andrews (1995) found that high elevation *Sceloporus scalaris* initiated reproduction and ovulated earlier than lowland populations. They attributed this response to time constraints at high elevation sites where embryonic development times are comparatively longer and the season is comparatively shorter.

As a result of the delay in ovulation of *N. ocellatus* at the Central Plateau, there is a corresponding delay in birth (late January to mid February) which results in an estimate of gestation length of the same range (90-105 days).The two study sites differ markedly in their climatic characteristics (see Chapter 2). Given this, the similar gestation length between the study sites is somewhat surprising, especially given the

relationship between basking opportunity and gestation length (Chapter 7 and references therein). Furthermore, parturition is delayed in gravid females from lowland sites transferred to subalpine enclosures compared to those transferred to lowland enclosures (unpublished data). Thus it was expected that gestation at the Central Plateau would take longer because of a presumed reduction in basking opportunities.

However, the thermal differences between the study sites are masked because gestation occurs over different time periods. At the Orford study site gestation occurs primarily during October, November and December, while at the Central Plateau it occurs primarily during November, December and January. Temperatures at the Central Plateau during the summer months of December and January are very similar to the temperatures during the spring months of October and November at Orford (Figure 2.4, Chapter 2). Furthermore lizard activity may be affected by both ambient air temperature and solar radiation. Levels of solar radiation during the summer months at the Central Plateau are higher than on the East Coast during spring and summer (Figure 2.7, Chapter 2), possibly providing gravid lizards with greater opportunities for basking, even when ambient air temperatures are relatively low.

The remarkable similarity in gestation length at the two sites has important implications for juvenile phenotype. Delays in parturition through prolonged gestation affect the phenotype and growth rate of neonate *N. ocellatus* in the laboratory (see Chapter 7 and references therein). Rapid gestation produces the largest (mass and length) and fittest (greatest growth rate) offspring. Therefore, the delay in ovulation at the Central Plateau and subsequent gestation through the warmest summer months may be important for the production of large, fit offspring. It is not known whether the delay in ovulation at the Central Plateau results directly from restricted activity time (and the resultant delay in follicular development) due to cool weather during late winter and early spring at the Central Plateau or from selection on female traits and behaviour that lead to later ovulation.

Interestingly, the only concession in the reproductive cycle of *N. ocellatus* to cold, subalpine climates appears to be a delay in the timing of the

reproductive events. In both populations 100 % of females reproduce each year and gestation is always completed within one season. This pattern is common in Australian skinks, but does not always occur. Some species do not reproduce every year, with the level of "skipped reproduction" related to climatic and food resources available each year (Schwarzkopf, 1992). Alpine species of *Niveoscincus* (*N. microlepidotus* and *N. greeni*) display an interesting adaptation to alpine conditions. These species display a biennial reproductive pattern, with gestation persisting for up to 14 months (Rawlinson, 1975; Hutchinson, Robertson and Rawlinson, 1989; Olsson pers. comm.). *Niveoscincus ocellatus* occurs sympatrically with both of these species at subalpine and alpine sites but does not show such a pattern.

There is evidence in other species that a bimodal reproductive pattern is possible within the same species, and that the length of gestation is controlled by environmental conditions. In the common gecko of New Zealand, *Hoplodactylus maculatus*, some populations have a five month annual gestation period, while those in cooler areas carry near full-term embryos over-winter, resulting in a fourteen month gestation, and a biennial reproduction (Cree and Guillette, 1995). This type of cycle differs from the much more common biennial reproduction involving "skipped reproduction" where ovaries remain largely inactive in non-reproductive years. An ability of the alpine *Niveoscincus* species to carry embryos over winter may well be an important adaptation that allows them to live in very cold areas. The generalist species, *N. ocellatus* and *N. metallicus*, do not appear to have this ability and this may prevent their occupation of areas occupied exclusively by *N. microlepidotus* and *N. greeni*.

3.4.2 Male and female gonadal cycles

Niveoscincus ocellatus exhibits a reproductive cycle in which males and females exhibit gonadal activity at different times of the year. Male *N. ocellatus* have a reproductive cycle similar to that of *N. metallicus* (Jones and Swain, 1996). In these species reproduction is characterised by prenuptial spermatogenesis, with predominantly autumn spermatogenesis and mating. This conforms to the Type II reproductive cycle recognised among Australian reptiles by Taylor (1985). Although it is sometimes difficult to accurately establish the timing of mating activity in small skinks (Greer, 1989) the clear copulation marks (see Figure 3.1 a and b) are readily identifiable in *N. ocellatus*. These marks are not present on any mature females during the latter half of pregnancy, so their first appearance in the following autumn (April/May) provides an accurate measure of the onset of mating behaviour and thus confirms the reproductive pattern first outlined for this species by Rawlinson (1974).

The mating grip in skinks has been little studied, probably due to their small size and cryptic nature (Greer, 1989), although mating marks have been previously described in a number of small Australian skinks including *Pseudemoia entrecasteauxii,* and *Pseudemoia spenceri* (Pengilley, 1972, cited in Greer, 1989). They provide an accurate estimation of the number of copulatory events in which a female has participated (Olsson, pers. comm.). The light coloured ventral surface of *N. ocellatus* also makes it possible to make judgements about the age of the mating marks, which is not always possible in other species (Swain and Jones, 1994).

In *N. metallicus* circumstantial evidence suggested that some animals may mate for a second time in spring (Swain and Jones, 1994). In the present study and Jones, Wapstra and Swain (1997) there are several lines of evidence to support a second period of mating in *N. ocellatus* . In *N. ocellatus* some females have fresh mating marks in late winter (August) with sperm in the distal oviducal lumina (Jones, Wapstra and Swain, 1997) in the Orford population. Furthermore, the majority of males caught during this time ejaculated motile sperm when their

hemipenes were everted and although their testes were small and regressed their epididymides were convoluted and white in appearance.

An asynchronous gonadal cycle in which spermatogenesis significantly precedes ovulation necessitates sperm storage by either males or females or both. When there are desynchronised gonadal cycles, as has been shown to occur in *N. ocellatus* and *N. metallicus* (Swain and Jones, 1994; Jones and Swain, 1996), the question of which sex stores the sperm relates to the issue of when mating occurs. For example in *Hemiergis decresiensis* from the Adelaide area males store the sperm in the vasa deferentia (Leigh pers. comm., cited in Hutchinson, 1993), while in the highlands the female may store the sperm (Pengilley, 1972, cited in Hutchinson, 1993). Therefore, sperm storage displays a high degree of lability in physiologically and behaviourally complex characteristics (Hutchinson, 1993).

The appearance of mating marks on all females in autumn and the presence of sperm in the oviducts indicate female sperm storage over the winter period. This is a common phenomenon in temperate lizards (e.g. Cuellar, 1966; Smyth and Smith, 1968). In *N. ocellatus*, as in most other lizards, sperm storage occurs in oviducal crypts (Jones, Wapstra and Swain, 1997). However, *N. ocellatus and N. metallicus* demonstrate an unusual pattern among Australian skinks, in which not only do females store sperm, but males may also store sperm in the epididymides providing a second "opportunity" to mate after the winter period of inactivity. During spermatogenesis the epididymides first begin to appear white and convoluted in appearance, and they maintain this appearance throughout the winter. Furthermore, during spring males ejaculate motile sperm, indicating an ability to provide viable sperm after winter. During this time, testes remain small and inactive.

Whether this period represents a true second period of mating activity, as occurs in many snake species (Saint Girons, 1985; Heatwole and Taylor, 1987) or simply is a reflection of a winter interruption to normal activity remains unclear. In snakes with an autumn and spring mating, spermatozoa transferred to the female during autumn remain in the lumen of the vaginal tube through winter, while spermatozoa used in

the spring mating are stored in the vasa deferentia of the male. In species displaying this pattern, this two phase insemination guarantees fertilisation of the clutch at the end of spring (Saint Girons, 1985). Viviparous South African cordylid lizards also mate in both autumn and spring, although in *Cordylus giganteus* the autumn mating is inferred rather than proven (Van Wyk, 1995), and in *Pseudocordylus melanotus* females may become pregnant in either autumn or spring (Flemming, 1993a). In *P. melanotus*, the testicular cycle is asynchronous with the ovarian cycle; maximum spermiogenesis occurs in late summer/early autumn, with ovulation in late winter/early spring. Successful reproduction in this species is ensured, however, by storage of spermatozoa by males in the epididymides through winter (Flemming, 1993a). Opposite asynchrony in reproductive cycles has been demonstrated in other viviparous lizards: *Sceloporus grammicus* (Guillette and Casas-Andreu, 1980), and *Sceloporus formosus* (Guillette and Sullivan, 1985), in which maximum testicular activity occurs in spring and vitellogenesis and ovulation occur in autumn.

Reptilian testicular cycles may be divided into two types (Saint Girons, 1982; Bradshaw, 1986; Van Wyk, 1995): those with prenuptial spermatogenesis, in which spermatogenesis immediately precedes mating, and those with postnuptial spermatogenesis, in which testicular recrudescence occurs after mating with subsequent sperm storage in the epididymides (termed an "estival cycle" by Saint Girons, 1985). Among temperate climate lizards, most lizards fall into the former category but postnuptial cycles are common in snakes and chelonians (Lofts, 1987). In *N. ocellatus*, testes size reaches a maximum in both populations during late summer/early autumn, prior to the onset of the autumn mating and this species may therefore be described as having prenuptial spermatogenesis.

Spermatogenesis is initiated in late summer when ambient air temperature is high. All reptiles studied to date require warm conditions for the final stages of testicular recrudescence (Licht, 1984) and Saint Girons (1985) suggests that spermatogenesis cannot occur at body temperatures below 20°C. The pattern described for the male gonadal cycle in *N. ocellatus* is in contrast with the cycle described for the

majority of temperate species in which testicular activity occurs during spring and early summer (Fitch, 1970; Duvall, Guillette and Jones, 1982, Licht, 1984; Lofts, 1987). Prior to the increase in testes size the epididymides appear small, unconvoluted, and clear. Soon after the testes begin to increase in size, the epididymides become convoluted and white, indicating storage of sperm prior to mating activity. There is a suggestion of a slight temporal displacement between the study sites. At Orford testes of male *N. ocellatus* begin to enlarge in November and reach their maximum size in January and February (Figure 3.5), whereas at the Central Plateau they do not begin to enlarge until December and reach their maximum size in February (Figure 3.6).

The observed plasma testosterone concentrations in male *N. ocellatus* from the Orford study site support the conclusions reached from examination of testes morphology. Males showed a bimodal seasonal cycle of plasma testosterone concentrations with a major peak in summer and autumn and a minor peak in August (Jones, Wapstra and Swain, 1997, Figure 3.7 this chapter). The major peak coincides with testicular activity of males at the Orford study site (Figure 3.5). Similar relationships between gonadal development and circulating concentrations of testosterone have been demonstrated previously (e.g. Arslan *et al.*, 1978). In species that have a prenuptial spermatogenic cycle there is usually a single peak in plasma androgens associated with maximum spermiogenesis (Lofts, 1987) and mating activity is usually associated with high concentrations of plasma androgens (Moore and Lindzey, 1992). However, when spermiogenesis and mating occur at different times, the temporal relationship between high plasma androgen concentrations and male mating behaviour may become uncoupled (Crews, 1984; Crews and Moore, 1986; Moore and Lindzey, 1992).

The association between mating behaviour and circulating concentrations of testosterone in reptiles is discussed further in Jones, Wapstra and Swain (1997) who suggest that the major peak in testosterone concentrations in *N. ocellatus* is associated with spermatogenesis and gonadal activity and that the second minor peak may be associated with mating activity in spring. Alternatively, mating

activity may be possible when testes are regressed and plasma concentrations of androgens are low; in such cases mating is thought to be triggered by rising temperature (Krohmer, Grassman, and Crews, 1987; Moore and Lindzey, 1992).

Niveoscincus ocellatus clearly exhibits asynchrony between male and female gonadal cycles. Follicular recruitment begins in late April in both populations with follicles showing some early signs of vitellogenesis before winter. Vitellogenesis continues through autumn and winter, when the testes are regressing in the males. Ovulation coincides with minimum testes size. One advantage of a reproductive pattern in which the sexes show asynchronous gonadal activity is that reproduction may be completed earlier (Guillette, 1982). Spermatogenesis requires a minimum of 8-10 weeks of favourable conditions in which males are able to maintain relatively high body temperatures (Saint Girons, 1985). If this period were to commence only after winter emergence then mating activity would not be possible until late spring, with a consequent delay in gestation. Given that this species is viviparous and that gestation lasts a minimum of 3 months, any such delay would endanger successful annual reproduction.

Vitellogenesis on the other hand is thought to be less demanding thermally and may proceed through the cooler months (Saint Girons, 1985). Asynchrony of the kind described in *N. ocellatus* has several advantages. It ensures adequate time for gestation with the young being born well before the end of the active season. As a result the potential for neonatal growth before winter is maximised (see also, Guillette and Méndez-de la Cruz, 1993).

During vitellogenesis plasma estradiol concentrations are high and peak prior to ovulation (Jones, Wapstra and Swain, 1997; Figure 3.3 this chapter). This is consistent with other reptile studies (e.g. Bona-Gallo *et al.*, 1980; Carnevali *et al.*, 1991; Jones *et al.*, 1983; Mead, Eraschenko and Highfill, 1981) including *N. metallicus* (Jones and Swain, 1996). There is no association between sexual activity and high concentrations of ovarian steroids or maximal levels of gonadal development. Plasma concentrations of progesterone were elevated during gestation in

N. ocellatus (Jones, Wapstra and Swain, 1997; Figure 3.4, this chapter); this is the norm in viviparous species (Yaron, 1985). Progesterone is thought to be synthesised primarily in the corpora lutea (Xavier, 1987); corpora lutea were visible throughout gestation in *N. ocellatus* but regressed rapidly after parturition. Swain and Jones (1997) suggest that circulating concentrations of progesterone may be linked to placental activity in *N. metallicus*. During gestation there is no evidence of follicle recruitment or vitellogenesis, which is thought to be due to the inhibiting action of progesterone. Further discussion of patterns of plasma progesterone concentrations in reptiles and the role of this hormone in the maintenance of gestation may be found in Jones, Wapstra and Swain (1997).

3.4.3 Annual variation in abdominal fat body weights

The study of energy stores and utilisation in relation to environmental variables can provide valuable information for understanding reptilian life histories (Taylor, 1986b). In this study *N. ocellatus* displayed a distinct annual cycle in stored lipids in the form of abdominal fat bodies. Lipid stores in temperate zone lizards cycle annually and are believed to serve as an energy source for winter dormancy and/or reproduction (Goldberg, 1974; Derickson, 1976; Flemming, 1993a; 1993b; Smith, Ballinger and Rose, 1995), but food availability is considered to be the ultimate factor determining whether or not lipids are stored (Derickson, 1976; Ballinger, 1977; Dunham, 1981). Derickson (1976) described four types of lipid cycling in reptiles. These patterns include; no lipid cycling, cycling associated only with winter dormancy, cycling associated with reproduction and cycling associated both with winter dormancy and reproduction. *Niveoscincus ocellatus* conforms to the pattern previously described for other Australian skinks: *Ctenotus taeniolatus* (Taylor, 1986b) and *Hemiergis decresiensis* (Robertson, 1981), in which lipid cycling is associated with both with reproduction and winter dormancy.

Niveoscincus ocellatus at Orford and the Central Plateau show a remarkably similar patterns of fat storage in both males and females. Small amounts of stored lipids by females after winter and through

spring are likely to be a reflection of the use of stored energy during winter and vitellogenesis. Decreases in fat stores have been recorded previously in many lizards during vitellogenesis, presumably because stored energy is diverted into follicle development (e.g. Marion and Sexton, 1971; Saint Girons, 1985; Lee *et al.*, 1989; Ramirez Pinilla, 1991; 1995; Smith, Ballinger and Rose, 1995). An increase in stored lipids during the late stages of pregnancy in *N. ocellatus* is thought to reflect both ready availability of energy (food) and a low energetic cost of maintaining the embryos. The majority of energy diverted to embryogenesis is invested in vitellogenesis, although there is evidence that there may be some maternal supplementation of embryo provisioning during pregnancy (Swain and Jones, 1997).

Increases in stored lipids during late pregnancy are somewhat unexpected given the high metabolic costs and/or reduced food intake often associated with pregnancy (e.g. Birchard *et al.*, 1984; Beuchat and Vleck, 1990; DeMarco and Guillette, 1992; DeMarco, 1993; Schwarzkopf, 1996a) and increases in the cost of movement (see Chapter 8 and references therein). However, early pregnancy may involve some energetic costs to the female, since they appear unable to increase fat reserves at this time, while males concurrently increase theirs. Presumably food is equally available to both sexes during this time and no differences in diet or food intake between the sexes were identified in the Orford population (Wapstra and Swain, 1996).

Male *N. ocellatus* show a different pattern of fat deposition to females. Fat bodies are small during spring but increase during summer. During this time males show no gonadal or other reproductive activity. Fat body mass decreases in males at the Orford site (unfortunately no male lizards were caught at the Central Plateau during this time) during the period in which gonadal activity is maximum (January - March) and continues to decrease during the mating period. Small fat bodies during winter presumably reflect energy usage during winter, and increases during early summer months are associated with high food availability and low levels of energy diverted to reproduction. As in other species, decreases in late summer/early autumn are associated with the period of maximal gonadal activity and the beginning of the mating season (e.g. Van Loben

Sels and Vitt, 1984; Rocha, 1992; Ramirez Pinilla, 1995) however, decreases in fat reserves are not always associated with spermatogenesis (Flemming, 1993b). Interestingly, fat bodies are already small in the Orford population at the beginning of winter (late June, Figure 3.9) raising the possibility that energy stores are more important for reproductive activity than survival over winter.

Chapter 4

Geographic and annual variation in reproductive output in *Niveoscincus ocellatus*

4.1 Introduction

Life history traits differ among species of lizards and often vary within populations. This variation occurs at several levels:

1. among species or higher taxa (see reviews by Stearns, 1984; Dunham and Miles, 1985b; Dunham, Miles and Reznick, 1988; Miles and Dunham, 1992);
2. among populations of geographically widespread species (e.g. Dunham, Miles and Reznick, 1988; Ferguson and Talent, 1993; Grant and Porter, 1992; Niewiarowski, 1994); and
3. annually within the same population (e.g. Dunham, 1978; Ballinger and Congdon, 1980; Schwarzkopf, 1992; Smith, Ballinger and Rose, 1995).

In this chapter variation in key life history characteristics of *N. ocellatus* is investigated at two levels: between populations (Orford and Central Plateau) and between years within each of these populations. While intraspecific variation in life history traits between populations may be associated with either genetic or environmental factors (these are investigated experimentally in Chapters 6 and 7), annual variation within populations is likely to reflect proximate effects on life history traits. Understanding these proximate factors may provide a greater understanding of the relative roles of proximate and ultimate factors in shaping life history traits (Smith, Ballinger and Rose, 1995).

Life history models suggest that at any point in an individuals' life there is an optimum proportion of available energy that the individual should divert to reproduction (Williams, 1966b; Stearns, 1976; Forsman and

Shine, 1995). The amount of energy diverted to reproduction is thought to be determined by trade-offs, particularly between current reproduction and future reproduction (Shine and Schwarzkopf, 1992; Forsman and Shine, 1995). Life history traits, such as the level of reproductive investment, should evolve so as to maximise the total lifetime fecundity of an individual (Shine and Schwarzkopf, 1992).

Any two components of the triad of clutch size, female reproductive investment, and expenditure per progeny uniquely determine the third. However, the forces of natural selection that mould each of these differ substantially. Clutch or litter weight presumably reflects an adult female's best current investment tactic in a given environment at a particular time, whereas expenditure on any given progeny is probably more closely attuned to the average environment encountered by a juvenile. Clutch or litter size is then the direct result of the interaction between reproductive investment by an individual lizard and an optimal offspring size, and clutch size is simply the ratio of the former divided by the latter (Heatwole and Pianka, 1993; Gregory and Larsen; 1996). In this chapter, the relationships between these three life history traits are investigated.

All models of the evolution of family size assume that there is a trade-off between the number of progeny produced and the fitness of each of them (Lessells, 1991; Roff, 1992; Stearns, 1992). This trade-off is expected on the basis that reproductive effort is usually divided between the offspring, so that the number of offspring produced can only be increased at the expense of the fitness of each of them (Lessels, 1991). This has long been recognised: Lack (1947) proposed that the optimal clutch size is determined by the trade-off between the number and fitness of the offspring. The evolution of offspring size has received considerable attention in the theoretical literature (e.g. Smith and Fretwell, 1974; Brockelman, 1975; Parker and Begon, 1986; Lloyd 1987; McGinley, Temme and Geber, 1987; Roff, 1992; Stearns, 1992). Recently, experimental approaches (e.g. Sinervo 1990a; Sinervo and Huey, 1990; Sinervo *et al.*, 1992; Landwer, 1994) have led to greater understanding of the selective forces shaping the trade-off between offspring size and number.

Offspring size is an important determinant of fitness because size at birth can influence growth rate, competitive ability, predator avoidance and survival, particularly during periods of environmental stress (e.g. Ferguson and Fox, 1984; Roff, 1992; Stearns, 1992). Offspring size may influence adult body size. For example, Gregory and Prelypchan (1994) found that variance in size at birth of garter snakes, *Thamnophis elegans*, contributed significantly to variance in adult body size. Similarly, Kaplan (1980) suggested that differences in the size of hatchling salamanders may become amplified over time so that hatchlings that are born larger remain larger at all stages of their life cycles. Larger offspring are thought to have a competitive advantage over smaller offspring in their cohort (Ferguson and Fox, 1984), although this is not always the case (Olsson, 1992; Sinervo *et al.*, 1992). Different environments may favour the selection of different sized offspring, through differential selective pressures on growth or survival. Selective pressures on offspring size will affect the trade-off between offspring number and offspring size for any given reproductive investment.

In this chapter four specific questions are addressed in relation to variation in life history traits between populations of *N. ocellatus* at Orford and the Central Plateau:

1. Is there a difference in adult body size at the two study sites?
2. What are the consequences of any size differences on other reproductive traits, such as clutch mass and number of offspring produced?
3. Do females invest equally in reproduction at the two study sites?
4. Do females from the two study sites apportion the energy invested in reproduction differently in terms of number and size of offspring (ie is there a trade-off between number and size of offspring between sites?).

Three specific questions are addressed in regard to variation in reproductive characteristics at each site:

1. Is there annual variation in reproductive investment?
2. Is the relationship between female size and number of offspring consistent?
3. Is there a trade-off between number and size of offspring?

4.2 Materials and methods

Lizards were collected from the two study sites (Chapter 2) at regular intervals for description of reproductive cycles (Chapter 3) and for further experimental work (Chapters 6, 7, and 8). They were brought to the laboratory where the following parameters were measured: snout vent length (SVL)(± 1 mm); total length (± 1 mm); and, if present, position of tail break (± 1 mm). They were sexed by eversion (if present) of hemipenes. Any animals not required for further experiments were either released at the site of capture or killed by placing them in the freezer at -20 °C (Cogger, 1992).

To examine reproductive characteristics, adult females were collected from the two sites throughout the reproductive season. Clutch size was determined in three ways. During the season it was determined either by autopsy of lizards or by abdominal palpation. Predictions of clutch size based on palpations were checked on a subsample and found to be 100% reliable for the Orford population, but were unreliable for the Central Plateau population because of the larger clutch sizes. At autopsy, the number of enlarged follicles or eggs/embryos in each oviduct, and the number of corpora lutea in each ovary were counted. Although rates of atresia of vitellogenic follicles are low in *N. ocellatus* (0.37% in the Orford population (Jones, Wapstra and Swain, 1997) data on clutch sizes based on predictions from early vitellogenic follicles in the reproductive cycle are not included, however, enlarged follicles in ovaries prior to ovulation are included.

The third method by which clutch size was determined was from live births in the laboratory. Collections of near-term gravid females were made late in the reproductive season in all three years. Females were held in small groups (2-4) in plastic terraria (20 x 30 x 10 cm) in the laboratory under standard conditions (14L:10D and 12 hour access to heat source) until parturition. Cages were checked daily for newborns. Females that had given birth were removed from their cage and weighed (± 0.1 mg) and measured (± 1 mm). Females were palpated to confirm that parturition was completed. Newborns were also weighed (± 0.1 mg) and measured (SVL, ± 0.05 mm; total length, 0.1 mm). A measure of their body proportion was obtained by dividing total body length by SVL and a measure of condition at birth (mg/mm) by dividing initial mass by SVL. In 1997 newborns were also sexed within three days of birth (see Chapter 6 for details). Offspring were either used in growth rate investigations (Chapter 6) or released at the site of maternal capture. Clutch mass (CM) is defined as the total mass of the offspring, and relative clutch mass (RCM) is defined as:

RCM = mass of clutch/mass of postpartum female.

Relative clutch mass (RCM) can be calculated as the ratio of clutch mass to either maternal gravid mass (clutch plus body), or post-gravid mass. The former method, although widely used (e.g. Vitt and Congdon, 1978; Vitt and Price, 1982), may introduce statistical artefacts because clutch mass is included in both numerator and denominator (Shine, 1980; Sinervo, Hedges and Adolph, 1991; Forsman and Shine, 1995). For this reason it has not been used here.

Farr and Gregory (1991) suggest that holding reptiles (snakes) in captivity can affect neonate phenotype and recommend that gravid reptiles be held for as short a time as possible so that variation in factors that influence neonate phenotype, including feeding level, temperature and stress, are minimised. Data on clutch mass and offspring size and mass were taken only from those lizards that had been in captivity for less than two weeks. Gravid lizards from Orford were consequently collected in late December/early January and from the Central Plateau in late

January/early February. However, in the first season of the study (1994/1995), parturition dates in the field were not known and females were held for slightly longer periods.

In the final season of the study (1996/1997) the trade-off between the size and number of offspring at each site was investigated using the residuals of the regression of female size and clutch size and using these in a subsequent regression with the average mass of offspring within each clutch (see Ford and Seigel, 1989b; Schwarzkopf, 1992; Olsson and Shine, 1997).

4.3 Results

4.3.1 Adult body size

Table 4.1 summarises the snout vent length (SVL) of adult male and female lizards from Orford and the Central Plateau. Females were judged to be mature by the presence of enlarged follicles or embryos during the reproductive season. Males were judged to be mature based on a comparison with sizes of known mature individuals derived from autopsy data (Chapter 3).

Table 4.1. Mean adult snout vent length at Orford and the Central Plateau (CP) for 1994/1995, 1995/1996, 1996/1997. Values are means ± standard error; sample sizes are indicated in parentheses.

	Female		Male	
Season	**Orford**	**CP**	**Orford**	**CP**
1994/1995	62.2 ± 0.64 (48)	73.1 ± 0.77 (46)	62.6 ± 0.99 (15)	70.9 ± 0.58 (38)
1995/1996	61.9 ± 0.47 (50)	73.5 ± 0.541 (105)	60.6 ± 0.97 (9)	72.2 ± 0.73 (46)
1996/1997	63.1 ± 0.27 (189)	71.3 ± 0.72 (51)	63.2 ± 0.50 (51)	72.9 ± 0.86 (23)
pooled data	62.7 ± 0.22 (290)	72.9 ± 0.38 (202)	62.8 ± 0.42 (75)	71.9 ± 0.42 (107)

The distribution of SVLs for reproductively mature female lizards from Orford for each of the years is shown in Figure 4.1 (a-c) and for the Central

Plateau in Figure 4.2 (a-c). There was no difference in the size of female lizards between years at either site (ANOVA: Orford $F_{2,284} = 2.526$; $P > 0.05$; CP $F_{2,199} = 2.803$; $P > 0.05$). Figure 4.3 displays the pooled data for female size for both sites. There was a significant difference in mature female size between sites (ANOVA $F_{1,487} = 587.893$; $P < 0.001$); females from the Central Plateau are larger, both at first reproduction and maximum size. The minimum SVL of mature females at Orford was 53 mm; however, a SVL of 55-57 was more typical of small mature females. The minimum SVL of mature females at the Central Plateau was 62 mm; however, a SVL of 65 mm was more typical of small females 65. Smaller females lizards were captured at each site, but these were immature.

SVL of mature males did not differ between years at either site (ANOVA: Orford; $F_{2,72} = 2.135$; $P > 0.1$; Central Plateau; $F_{2,104} = 1.692$ $P > 0.1$) Pooled data for each site are presented in Figure 4.4. Mean SVL of adult males differed significantly between sites (ANOVA: $F_{1,180} = 221.703$; $P < 0.001$). Similarly to females, males at the Central Plateau are larger at sexual maturity and reach a larger maximum size than those from Orford. The minimum size at maturity for males was similar to that of females at both sites; SVL of 54-55 mm at Orford and 62-64 mm at the Central Plateau.

Male and female SVL did not differ at either site (ANOVA: Orford; $F_{1,360} = 0.003$; $P > 0.1$; Central Plateau $F_{1,307} = 2.417$; $P > 0.1$). Mean adult sizes were 62.7 ± 0.20 mm at Orford and 72.5 ± 0.29 mm at the Central Plateau. Adult male and female lizards could not be divided into age classes based on their size (Figures 4.3 and 4.4), although it is likely that the smallest lizards represent the youngest cohort, and progressively larger lizards older cohorts. Relationships between size and age within each site and differences between sites are investigated in more detail in Chapter 5.

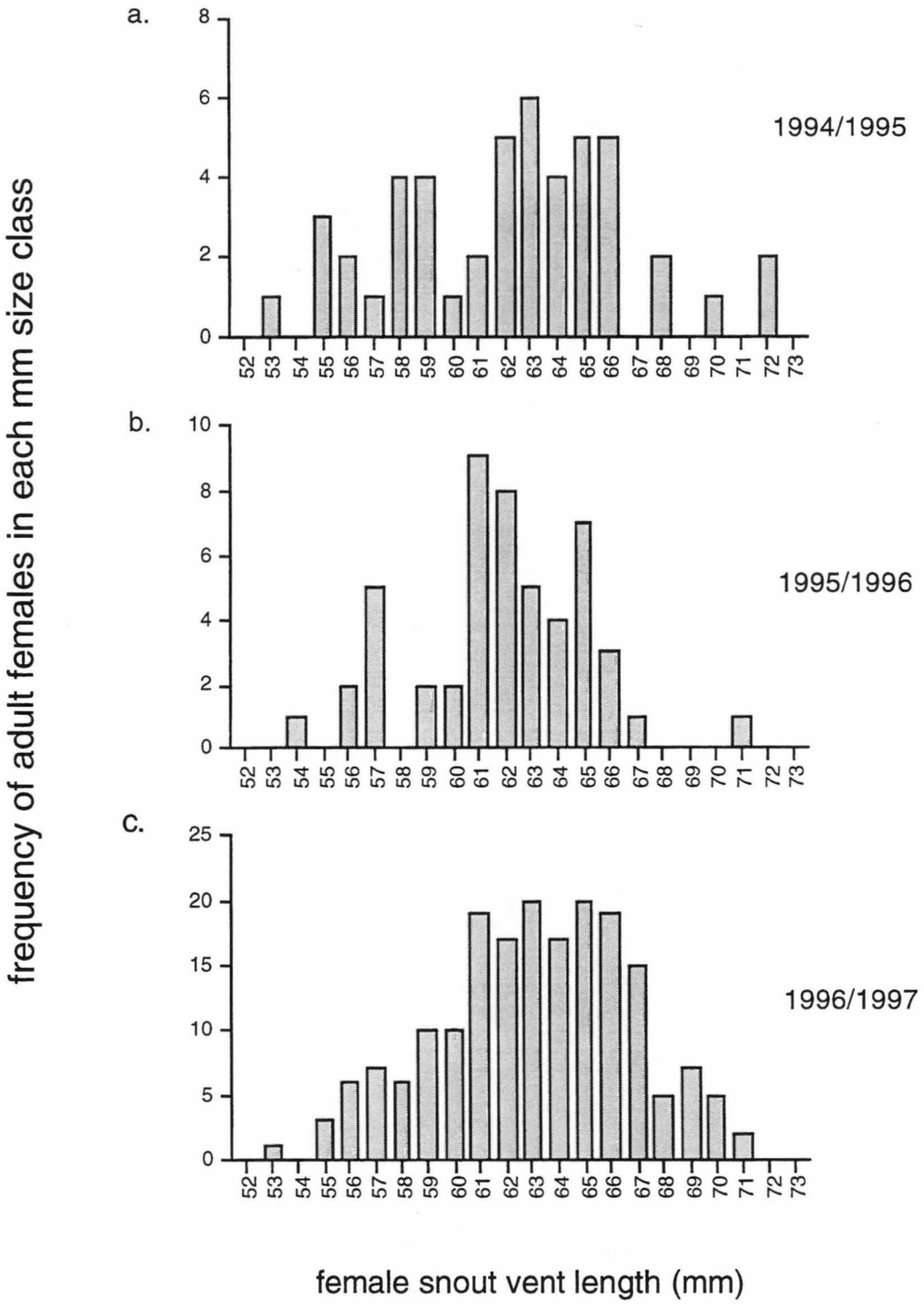

Figure 4.1. Size frequency distribution in snout vent length for adult female *Niveoscincus ocellatus* at Orford for three seasons (1994/1995, 1995/1996, 1996/1997).

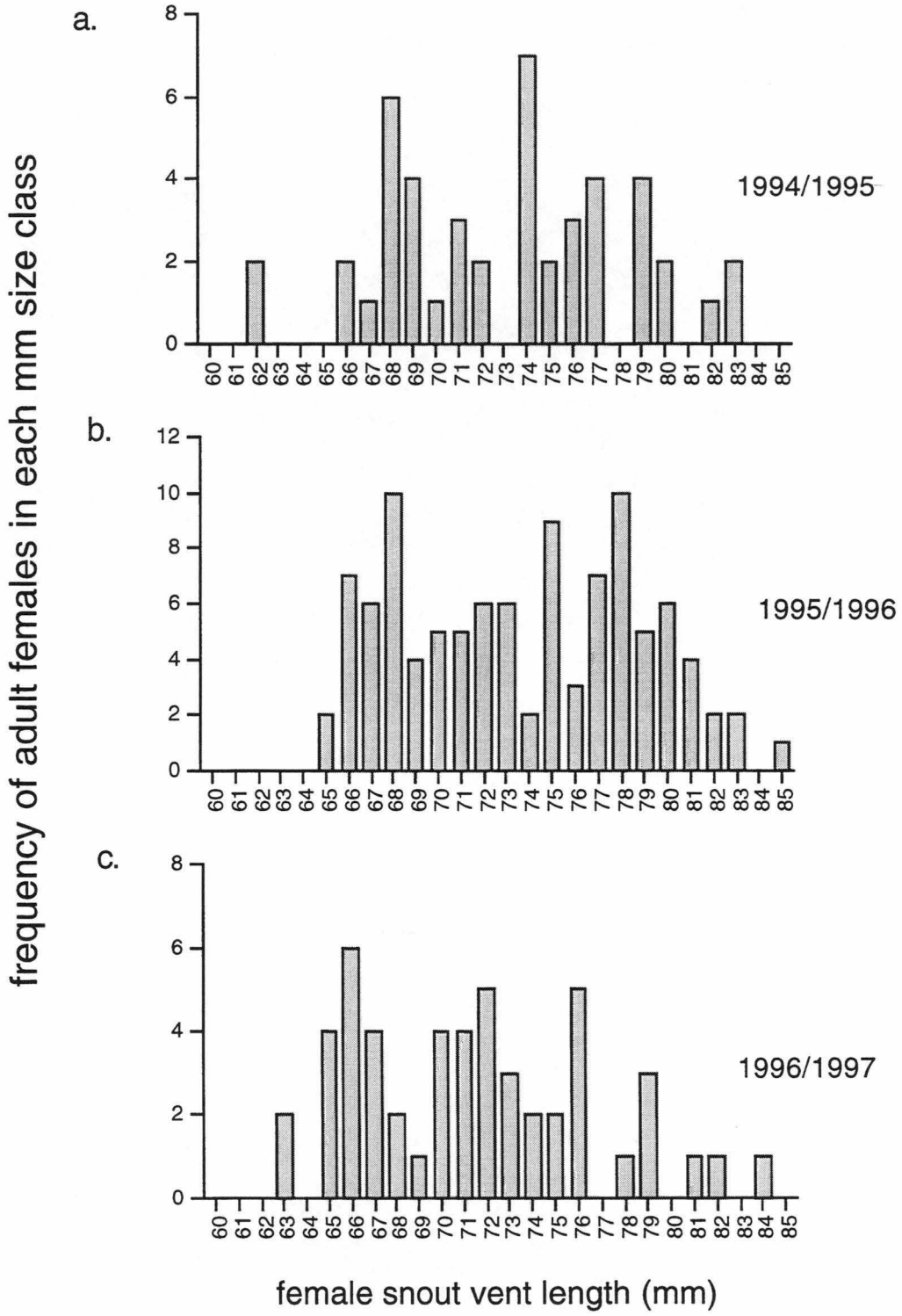

Figure 4.2. Size frequency distribution in snout vent length for adult female *Niveoscincus ocellatus* at the Central Plateau for three seasons (1994/1995, 1995/1996, 1996/1997).

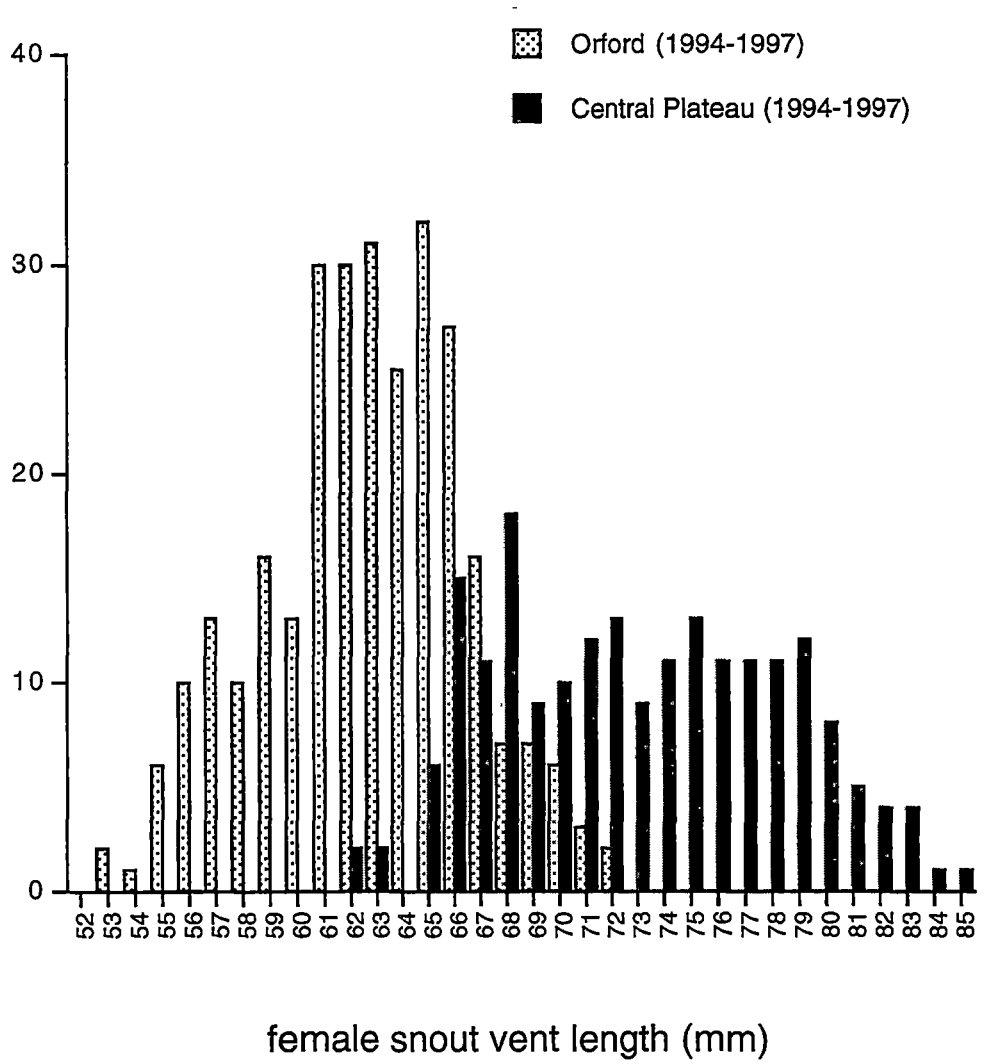

Figure 4.3. Size frequency distribution in snout vent length for adult female *Niveoscincus ocellatus* at Orford and the Central Plateau during 1994-1997.

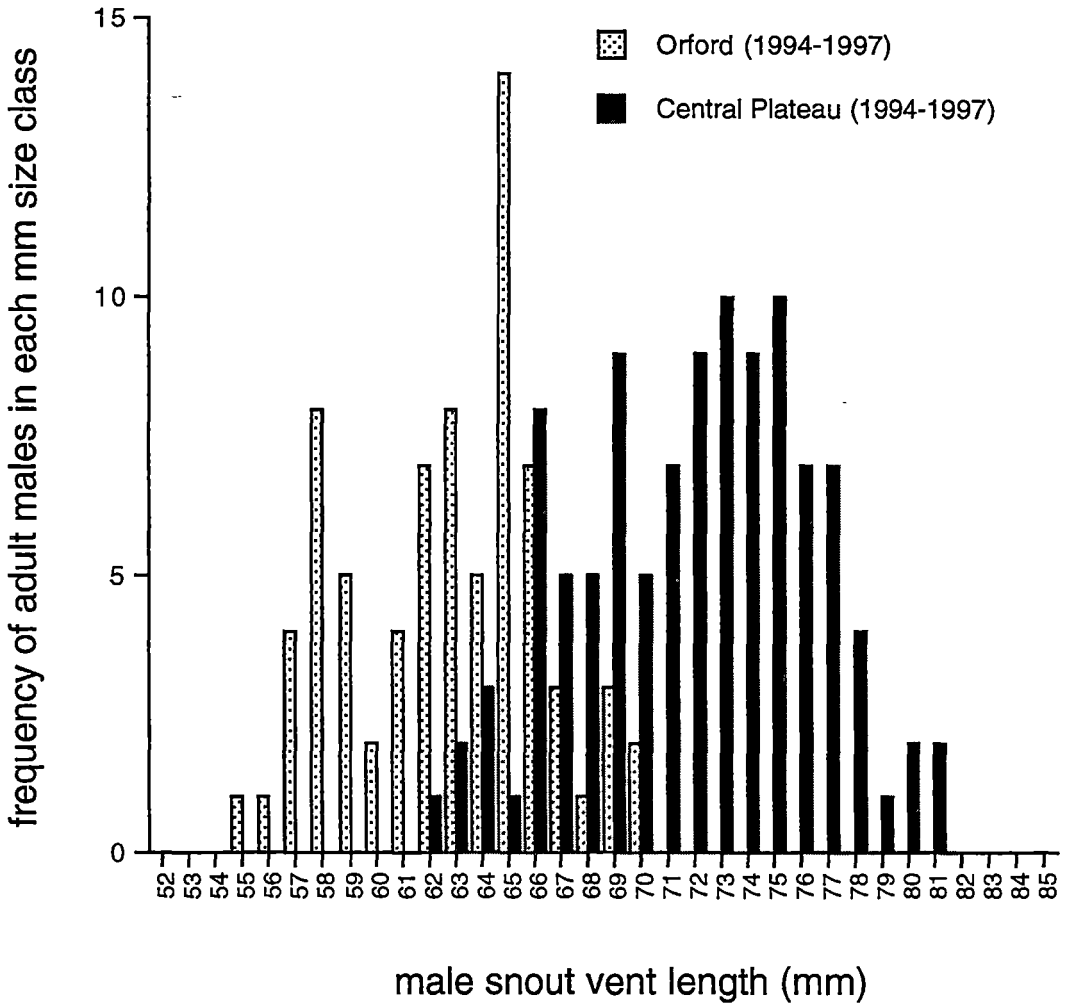

Figure 4.4. Size frequency distribution in snout vent length for adult male *Niveoscincus ocellatus* at Orford and the Central Plateau during 1994-1997.

4.3.2 Clutch characteristics

Clutch characteristics for all three seasons at both sites are shown in Table 4.2. Clutch mass does not differ at the Central Plateau between years (ANOVA: $F_{2,52} = 0.631$; $P > 0.1$), but there was significant variation between years at Orford (ANOVA: $F_{2,127} = 3.709$; $P = 0.027$). In both populations, clutch mass was positively correlated with female size in all years ($P < 0.01$), but RCM was unrelated to female size ($P > 0.05$). Clearly clutch mass differs between the study sites, with females from the Central Plateau having a greater clutch mass than those from Orford (approximately 40%). However, this large clutch mass is largely attributable to the increased female body size and an increase in the number of offspring produced (see below).

Relative clutch mass (RCM) differed at Orford between the years of 1995/1996 and 1996/1997 (ANOVA: $F_{1,89} = 16.116$; $P < 0.001$) but did not differ between years at the Central Plateau. The low RCM of the Orford population in 1995/1996 resulted in it differing from the Central Plateau population (ANOVA: $F_{1,61} = 8.361$; $P < 0.01$) but there was no difference between the sites in 1996/1997 (ANOVA: $F_{1,110} = 0.093$; $P > 0.1$).

Table 4.2. Average clutch masses for *Niveoscincus ocellatus* from Orford and the Central Plateau (CP) study sites during the three year study period. Values are means ± standard error. Sample sizes are indicated in parentheses.

	clutch mass (mg)		RCM	
year	Orford	CP	Orford	CP
1994/1995	1126.4 ± 58.18 (32)	1960.2 ± 249.52 (12)	na	na
1995/1996	1076.0 ± 55.09 (26)	1836.5 ± 177.56 (16)	0.23 ± 0.011 (21)	0.27 ± 0.021 (15)
1996/1997	1246.1 ± 35.48 (72)	2089.3 ± 126.14 (27)	0.29 ± 0.009 (71)	0.30 ± 0.018 (27)

The mean clutch size for each population is presented in Table 4.3.

Table 4.3. Mean clutch size of *Niveoscincus ocellatus* at Orford and the Central Plateau for 1994/1995, 1995/1996, and 1996/1997. Values are means ± standard error. Sample sizes are indicated in parentheses.

year	Orford	Central Plateau
1994/1995	2.4 ± 0.10 (51)	4.3 ± 0.27 (36)
1995/1996	2.3 ± 0.10 (50)	3.9 ± 0.15 (66)
1996/1997	2.6 ± 0.05 (185)	3.7 ± 0.14 (46)

There is a difference in mean clutch size between years at Orford (ANOVA: $F_{2,283} = 4.215$; $P = 0.016$); however when clutch size is adjusted for differences in female body size there are no significant differences between years (ANCOVA: $F_{2,279} = 1.874$; $P > 0.1$). In all results from ANCOVA, any differences reported represent differences in Y-intercepts of the regression of female size and clutch size. Differences in slope represent a significant interaction effect, a violation of the assumptions of ANCOVA (Sokal and Rohlf, 1981; Wilkinson, 1989). Figure 4.5 illustrates the relationship between female size and clutch size for the Orford population for three years. There were no differences in the slopes of the regression of female size and clutch size for the Orford population for the three years ($F_{2,277} = 0.180$; $P > 0.5$). The data were pooled and provided a common regression equation of:

$$y = 0.109x - 4.349; r = 0.610; P < 0.001,$$

where y = clutch size and x = female SVL.

Mean clutch size in the Central Plateau population did not vary during the three year study (ANOVA: $F_{2,145} = 2.489$; $P = 0.086$) (Table 4.3). There was a positive relationship between female size and clutch size in all years (Figure 4.6). The equations describing the relationship are:

1994/1995; y = 0.262x - 14.892; r = 0.882; $P < 0.001$:
1995/1996; y = 0.176x - 9.045; r = 0.738; $P < 0.001$
1996/1997; y = 0.104x - 3.771; r = 0.882; $P < 0.001$.

There are significant differences in the slopes of the regression lines ($F_{2,139} = 10.036$; $P < 0.001$). Figure 4.7 provides a visual comparison of the relationship between SVL and clutch size at the two sites.

Clutch size differs significantly between sites. Most of this difference is explained by the larger size of females at the Central Plateau. However, there is a significant difference in the slopes of the regression between Orford and the Central Plateau in 1994/1995 ($F_{1,315} = 60.078$; $P < 0.001$) and 1995/1996 ($F_{1,345} = 14.639$; $P < 0.001$). In these years the slope of the regression line derived for the Central Plateau population was greater than at Orford, implying that females from the Central Plateau produced relatively more offspring than those from Orford. In 1996/1997 there was no difference in slopes of the regression ($F_{1,322} = 0.056$; $P > 0.5$), and differences in mean clutch size could be analysed by ANCOVA. Clutch size differs between sites (ANOVA: $F_{1,330} = 109.224$; $P < 0.001$), but when analysed with female SVL as the covariate the difference in clutch size is only marginally significant ($F_{1,323} = 3.920$; $P = 0.049$) and reflects a slightly greater number of offspring produced for a given body size at the Central Plateau than at Orford (Figure 4.7).

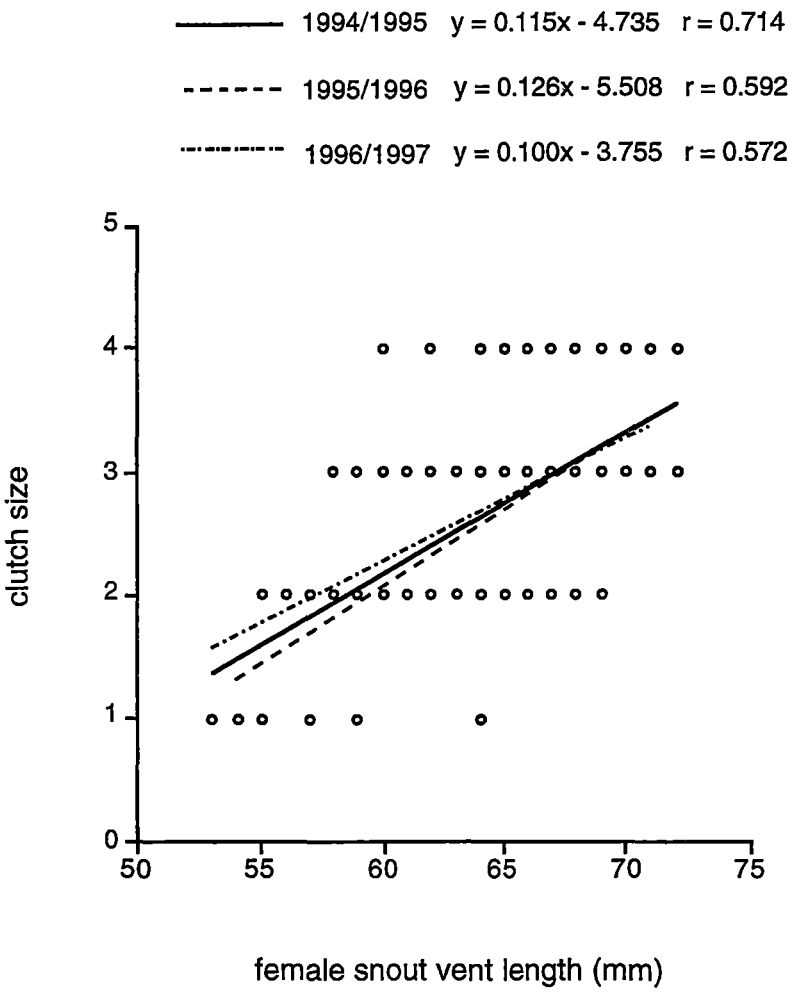

Figure 4.5. Relationships between snout vent length (mm) and clutch size of female *Niveoscincus ocellatus* for the Orford population.

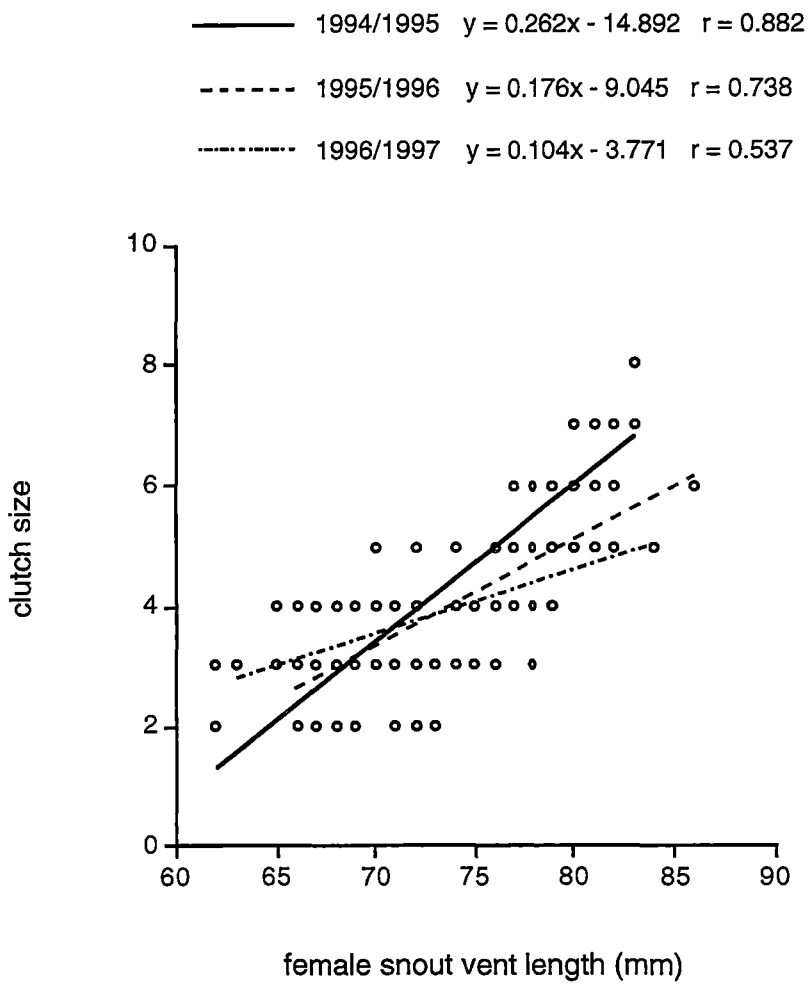

Figure 4.6. Relationships between snout vent length (mm) and clutch size for female *Niveoscincus ocellatus* for the Central Plateau population.

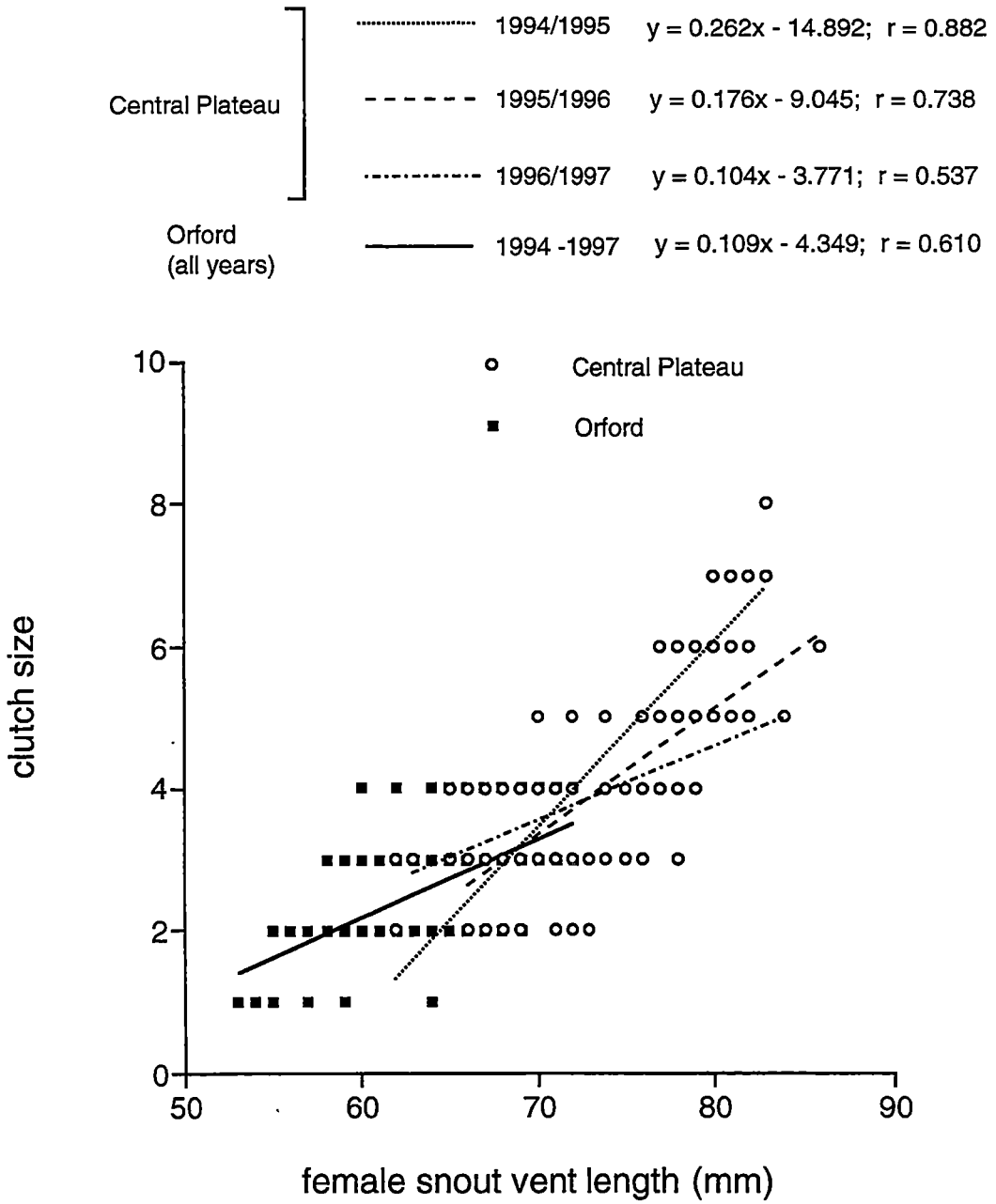

Figure 4.7. Comparison of relationships between maternal snout vent length and clutch size of female *Niveoscincus ocellatus* at Orford and the Central Plateau. Orford data pooled for 1994-1997. Slopes of regression lines for Central Plateau differ each year (see results).

4.3.3 Offspring characteristics

4.3.3.1 Annual variation at Orford and the Central Plateau

Table 4.4 summarises morphometric data for offspring born in the laboratory from near term gravid female *N. ocellatus* from Orford and the Central Plateau. Differences between years at each site were analysed and the results of the analyses are presented in Table 4.5. All offspring characteristics differed between years at both sites, with the exception of body proportions which was the same at the Central Plateau in all years. Body proportion was calculated as: total length (mm) / snout vent length (mm). In general, offspring were largest at Orford in the 1996/1997 season, both in length and weight and smallest in 1994/1995. Offspring from the Central Plateau were similarly larger in 1996/1997, but were smallest in 1995/1996 (Table 4.4).

Table 4.5. Summary statistics for differences between years in *Niveoscincus ocellatus* offspring characteristics at Orford and the Central Plateau.

trait	Orford		Central Plateau	
SVL	$F_{2,310}$ =47.515	$P < 0.001$	$F_{2,204}$ =48.744	$P < 0.001$
weight	$F_{2,310}$ =30.395	$P < 0.001$	$F_{2,207}$ =25.162	$P < 0.001$
total length	$F_{2,306}$ =42.805	$P < 0.001$	$F_{2,201}$ =19.448	$P < 0.001$
condition	$F_{2,310}$ =41.044	$P < 0.001$	$F_{2,204}$ =13.563	$P < 0.001$
proportion	$F_{2,307}$ =5.559	$P < 0.01$	$F_{2,202}$ =0.126	$P > 0.5$ ns

4.3.3.2 Within year variation between sites

Differences between sites were examined for each of the years, and consistent differences were identified, despite annual variation within sites. Housing conditions and collection dates of females differed between years (which may have affected offspring characteristics; Farr and

Table 4.4. Offspring characteristics of *Niveoscincus ocellatus* for the three seasons of study (1994/1995, 1995/1996, 1996/1997) from the Orford and Central Plateau study sites. Values are means ± standard error and sample sizes are indicated in parentheses. Condition of offspring represents their mass divided by SVL and proportion provides a measure of their relative tail length and is calculated as total length divided by SVL.

	SVL (mm)		total (mm)		weight (mg)		condition (mg/mm)		proportion (total/SVL)	
year	Or	CP	Or	CP	Or	CP	Or	CP	Or	Cp
94/95	28.3 ± 0.11	29.6 ± 0.09	65.0 ± 0.33	66.5 ± 0.33	460.9 ± 5.87	534.7 ± 7.66	16.3 ± 0.17	18.1 ± 0.23	2.3 ± 0.007	2.3 ± 0.007
	(78)	(59)	(78)	(59)	(78)	(59)	(78)	(59)	(78)	(59)
95/96	29.1 ± 0.10	29.3 ± 0.08	67.5 ± 0.32	65.9 ± 0.25	480.6 ± 6.13	517.5 ± 6.01	16.5 ± 0.18	17.6 ± 0.20	2.3 ± 0.007	2.3 ± 0.006
	(61)	(53)	(59)	(51)	(61)	(57)	(61)	(54)	(59)	(52)
96/97	29.4 ± 0.06	30.3 ± 0.06	68.3 ± 0.20	68.2 ± 0.23	512.6 ± 3.88	574.8 ± 5.05	17.4 ± 0.11	18.9 ± 0.15	2.3 ± 0.004	2.2 ± 0.005
	(174)	(94)	(172)	(94)	(174)	(94)	(174)	(94)	(172)	(94)

Gregory, 1991); however there were no differences in collection procedures within years between sites. Therefore comparisons of offspring characteristics between sites can be made for each year (Table 4.6).

Table 4.6. Summary statistics of differences in *Niveoscincus ocellatus* offspring characteristics between Orford and the Central Plateau for each season.

trait	1994/1995		1995/1996		1996/1997	
SVL	$F_{1,136} = 70.961$	$P < 0.001$	$F_{1,112} = 1.842$	$P > 0.05$ ns	$F_{1,266} = 75.957$	$P < 0.001$
total	$F_{1,135} = 10.480$	$P < 0.01$	$F_{1,108} = 13.868$	$P < 0.001$	$F_{1,264} = 0.212$	$P > 0.1$ ns
weight	$F_{1,135} = 60.548$	$P < 0.001$	$F_{1,116} = 18.391$	$P < 0.001$	$F_{1,266} = 93.008$	$P < 0.001$
condition	$F_{1,135} = 41.215$	$P < 0.001$	$F_{1,113} = 17.218$	$P < 0.001$	$F_{1,266} = 69.908$	$P < 0.001$
shape	$F_{1,136} = 20.874$	$P < 0.001$	$F_{1,109} = 47.784$	$P < 0.001$	$F_{1,264} = 105.526$	$P < 0.001$

Offspring from the Central Plateau were typically larger than offspring from Orford (except in 1995/1996 when SVL did not differ), both in length and weight. Offspring from the Central Plateau were approximately 10 % heavier than from Orford but were only about 3 % longer. Offspring from the Central Plateau were also in better condition (they were heavier for any given length) than those from Orford.

4.3.3.3 Trade-off between clutch size and offspring size

In 1996/1997 the trade-off between offspring size and clutch size within each population was also investigated. Only females that gave birth in the laboratory were used in the regression of female size and clutch size (Figure 4.8). The residuals from the regression were used in the subsequent regression with mean offspring mass (Figure 4.9). In both populations there was a significant negative correlation between offspring mass and the residuals:
Orford: $y = -41.595x + 516.244$; $r = 0.494$; $F_{1,70} = 22.579$; $P < 0.001$;
Central Plateau: $y = -38.540x + 562.731$, $r = 0.476$; $F_{1,24} = 7.044$; $P < 0.05$.

This indicates that there is a trade-off between number and size of offspring at each site; as females produce more offspring for a given size, the individual offspring produced are smaller.

4.3.3.4 Sex of offspring

In the final season (1996/1997) offspring were sexed (see Chapter 6 for methods). From the Orford population there were 174 offspring born, and of these 172 were sexed; 95 were male and 77 were female. From the Central Plateau population there were 94 offspring born and of these 93 were sexed; 51 were male and 42 were female. Although there were more male offspring, the ratio of male to female does not deviate significantly from a 1:1 ratio ($P > 0.1$ for both populations). There were no significant differences between male and female offspring from the Central Plateau ($P > 0.1$ for weight, SVL, total length and condition). Similarly, male and female offspring did not differ from the Orford population in SVL or total length ($P > 0.1$), but males were marginally heavier ($F_{1,170} = 3.431$ $P = 0.066$) and were also heavier for their length ($F_{1,170} = 4.028$ $P = 0.046$).

4.4 Discussion

4.4.1 Variation in adult body sizes between sites

In *N. ocellatus* there were distinct differences in adult body size at Orford and the Central Plateau. Lizards from the Central Plateau matured at a larger size and also reached a larger maximum size. There were no differences in the average size of adult male or female lizards at either site, and the mean size of adults at the Central Plateau (72.5 mm) was greater than the largest adult lizard caught at Orford (72 mm). Discussion in this chapter is restricted to the consequences of female size differences for reproductive output.

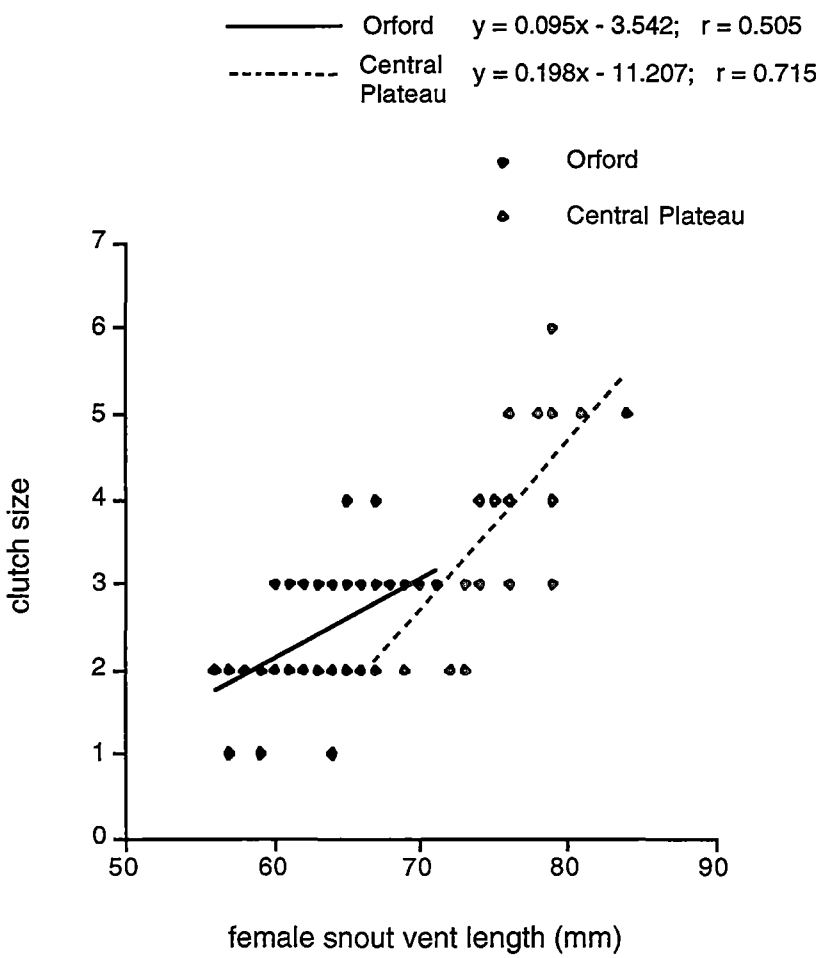

Figure 4.8. Relationship between snout vent length and clutch size for female *Niveoscincus ocellatus* at Orford and the Central Plateau which gave birth in the laboratory in 1996/1997. residuals from regression lines were used to analyse the trade-off between clutch size and offspring size (see Figure 4.9.).

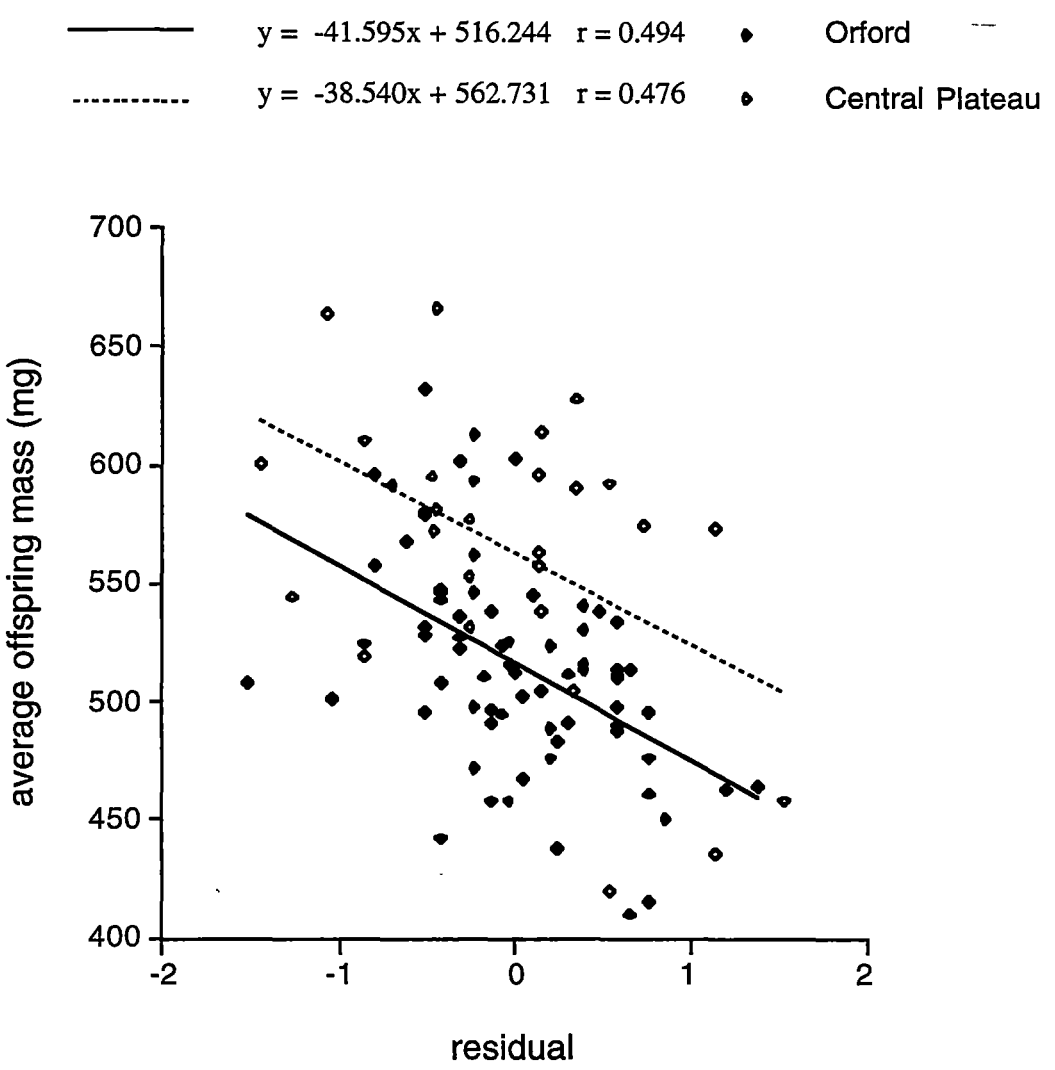

Figure 4.9. Relationship between mean offspring size and residuals of the regression of female snout vent length and clutch size (Figure 4.8.) of female *Niveoscincus ocellatus* at Orford and the Central Plateau.

Large adult body size at the Central Plateau may reflect a later age at maturity, or differences in growth patterns, or both. No distinct size classes were apparent in the distribution of adult body sizes at either site (although there were small individuals that were presumably breeding for the first time, and a smaller number of larger animals which presumably represent the oldest cohorts). Ageing *N. ocellatus* by size class is therefore not possible at either site. Chapter 5 provides a detailed investigation of the relationship between size and age of *N. ocellatus* by skeletochronology with an emphasis on differences in growth patterns and age at maturity at the two sites.

4.4.2 Reproductive output

In *N. ocellatus* fecundity in any year increases with increasing body size. Reproductive output (clutch mass and RCM) showed little variation between years and between sites. The only exception was the Orford population during 1995/1996 when clutch mass and RCM was reduced (compared to both the Central Plateau and other years at Orford), although clutch size remained the same (see below). Constant relative clutch mass is common in many lizards and snakes (e.g. Shine, 1980; DeMarco, 1989; Schwarzkopf, 1992; Tinkle, Dunham and Congdon, 1993; Olsson and Shine, 1997). For example, Schwarzkopf (1992) found that relative clutch mass (RCM) did not vary with year, age, or size of female for a small to medium sized skink, *Eulamprus tympanum* over a two year period. Similarly, Olsson and Shine (1997) found that RCM in *Lacerta agilis* stayed relatively constant throughout a five year study period, despite inter-annual differences in maternal size, mean clutch size, relative fecundity and mean hatchling mass.

The constancy of RCM compared to other reproductive variables, such as frequency of reproduction, clutch size, and offspring size, prompted DeMarco (1989) to conclude that perhaps it was RCM, rather than other features of life history, that was optimised by selection. Relative clutch mass is thought to be optimised through selection on body size and shape, foraging mode and/or escape tactics (Vitt and Congdon, 1978; Vitt

and Price, 1982). *Niveoscincus ocellatus* utilises similar active searching type foraging behaviour at both sites (Wapstra and Swain, 1996 and pers. obs.) and relies on flight to escape predators, indicating that selective pressures on RCM in relation to these factors are likely to be similar at both sites. The relationship between clutch mass, female body size, and sprint speed are investigated further in Chapter 8.

The low reproductive output in 1995/1996 in the Orford population may be attributed to the weather conditions during that period. The 1995/1996 season was a particularly poor season on the east coast of Tasmania, with high rainfall and cloud cover and lower than average temperatures. During this season, parturition occurred at a later date in the Orford population (Chapter 3), presumably due to reduced basking opportunities. Variation in reproductive output between either years or populations may result from a variety of factors. Jones, Ballinger and Porter (1987) proposed that, although food abundance or rainfall are commonly cited as the source of annual variation in reproductive traits (e.g. Ballinger, 1977; Dunham, 1981), other physical factors may also be important, particularly temperature and water availability. Temperature, particularly, may affect reproductive parameters, through its effect on potential activity time, food abundance, and foraging opportunities to lizards (James, 1991; Olsson and Shine, 1997).

4.4.3 Relationship between female size and clutch size

4.4.3.1 Annual variation

There have been few studies of annual variation in the reproductive characteristics of viviparous reptile species (Schwarzkopf, 1992). Documentation of such variation within populations of reptiles has helped to elucidate some of the constraints that influence life history tactics at a micro-evolutionary level (Schwarzkopf, 1992; Olsson and Shine, 1997). In species in which clutch frequency is fixed at one per year (which includes most snakes and viviparous lizards) variation in clutch size and/or offspring size occurs in response to environmental variation (Seigel and Fitch, 1985; Schwarzkopf, 1992).

In *N. ocellatus*, clutch size is positively related to female body size (SVL) in both populations. In reptiles, clutch size is generally (e.g. Tinkle, Wilbur and Tilley, 1970; Marion and Sexton, 1971; Congdon and Gibbons, 1985; Taylor, 1985; Seigel and Ford, 1987; Ford and Seigel, 1989b; Tinkle, Dunham and Congdon, 1993; Rowe 1994; Olsson and Shine, 1997), though not always (e.g. Goldberg, 1974; Guillette and Casas-Andreu, 1987; Howland, 1992) positively related to female size. A positive relationship between female size and number of offspring has been documented in other Australian skinks (e.g. Taylor, 1985; Schwarzkopf, 1992) including *Niveoscincus metallicus* (Jones and Swain, 1996).

In *N. ocellatus* there was no difference in the relationship between female size (SVL) and the size of clutch between years at the Orford site. Data were also available for the 1992/1993 season (Wapstra, 1993) and 1993/1994 season (Jones, Wapstra and Swain, 1997, unpublished data) and in these years the relationship between female size and clutch size was the same as for the following three years ($P > 0.1$). This relationship remains constant despite variation in reproductive output (see above) and offspring size (see below).

Theoretical predictions suggest that offspring size should remain relatively constant (strong selection on offspring size) while litter size may change in response to variations in resources (Smith and Fretwell, 1974; Brockelman, 1975; Lalonde, 1991; Forsman and Shine, 1995). However, in species in which clutch size is low, this is not always the case. For example, Schwarzkopf (1992) showed that there was no significant annual variation in clutch size in *Eulamprus tympanum* but there was significant variation in offspring size. Similarly, in a detailed examination of reproductive parameters in *Lampropholis delicata* across a wide geographic range, Forsman and Shine (1995) found that mean clutch size and mean egg size were equally variable, despite predictions that clutch size would be more variable than offspring size. Forsman and Shine (1995) argue that variation in offspring size (rather than offspring number) may result from the fact that offspring size is a continuous number, while clutch size is an integer, and thus only very coarse

adjustments are possible to clutch number, while finer adjustments may be made to offspring size (see also Stearns, 1992; Ebert, 1994).

In *N. ocellatus*, as in *E. tympanum* (Schwarzkopf, 1992), the slope of the relationship between female size and clutch size was not very steep (slope = 0.109) for the Orford population. Thus a large increase in female SVL (over 9 mm) is required before females are able to produce an extra offspring. In species which produce small litters that are strongly constrained by female size, small variations in energy availability and/or other exogenous variables affecting reproduction are therefore more likely to translate to variations in offspring size, rather than offspring number or frequency of litter production (Schwarzkopf, 1992; Ebert, 1994).

However, the relationship between female size and clutch size is more variable at the Central Plateau than at Orford. In the Central Plateau population clutch size is less constrained by female size than at Orford. An increase in female SVL of only 3.8 mm (1994/1995), 5.6 mm (1995/1996), and 9 mm (1996/1997) resulted in an increase in clutch size.

If resources vary between years, there are two possible outcomes, either the number of offspring is affected or the size of each offspring is affected (e.g. Lalonde, 1991). The outcome will depend on the amount of energy available for reproduction in that year and the minimum size for a viable offspring. Consider the following simplified case: at Orford the average clutch size is approximately 2.4 offspring of 500 mg each and clutch mass is 1200 mg, and at the Central Plateau it is 4 offspring of 500 mg each and the clutch mass is 2000 mg. A similar change in the amount of energy available to an individual female (from the average) at the two sites may result in different outcomes being made. For example, a 10 % increase in the energy available for reproduction will result in an increase of 120 mg in total clutch mass at Orford and 200 mg at the Central Plateau. In both cases, the average size of the offspring may be increased to 550 mg, or the clutch size may be increased. In the example the average mass per offspring in an increased clutch is 388 mg at Orford, while at the Central Plateau it is 440 mg. Assuming that the minimum size for a viable offspring is at least 400 mg (which is based on observations of births and

growth rates in the laboratory) then clutch size is likely to remain the same at Orford and offspring size will change, while at the Central Plateau, clutch size may change.

Previous studies of viviparous reptiles have demonstrated annual variations in litter sizes (e.g. Parker and Pianka, 1975; Reznick and Sexton, 1986; Seigel and Fitch, 1985; Smith, Ballinger and Rose, 1995) and variation in offspring size (Andren and Nilson, 1983; Brodie and Ducey, 1989; Schwarzkopf, 1992). Ford and Seigel (1989b), however, claimed that variation in offspring size, with no concomitant variation in litter size, is rare in reptiles, although, it has been observed in more recent studies (Schwarzkopf, 1992; Forsman and Shine, 1995; Olsson and Shine, 1997) and now in this study in *N. ocellatus* at Orford.

4.4.3.2 Interpopulational variation

Clearly, clutches are much larger at the Central Plateau. Typically, in intraspecific comparisons of squamate reptiles, differences in reproductive output are mostly attributable to variation in maternal size (e.g. Tinkle, Wilbur and Tilley, 1970; Ballinger, 1979). Although some of the variation in clutch size in *N. ocellatus* is a consequence of body size, this does not fully account for the observed variation in clutch sizes. In two of the three years (1995/1996 and 1996/1997), females from the Central Plateau produced relatively more offspring as their size increased (slope of regression greater than at Orford), and in 1996/1997 Central Plateau females produced more offspring for their size than females at Orford, although the slopes of the regressions were the same (Figure 4.7). This is despite the fact that Central Plateau females also produced larger young (see below). This is contrary to the finding of Sinervo (1990b) who demonstrated a phenotypic trade-off between egg size and clutch size in *Sceloporus undulatus* between populations; high elevation populations produced more young that were smaller.

Similarly, Forsman and Shine (1995) found that variation in reproductive traits between geographically widespread populations of

Lampropholis delicata was not a simple consequence of variation in body size among populations, because significant interpopulation differences were still evident after the effects of maternal snout vent length were accounted for. The rate at which fecundity increased with maternal size varied among populations and showed the greatest increase in the southern most population (and presumably the coldest site). In *N. ocellatus*, a comparable trend is evident: clutch size increases more rapidly with maternal size at the colder Central Plateau site. In contrast to my study, and that of Forsman and Shine (1995), Rohr (1997) found that low altitude female *Eulamprus tympanum* had higher size specific reproductive output than high altitude females. Rohr (1997) concluded that the relatively low reproductive output in the high altitude population was a result of greater resource limitation. Female *N. ocellatus* from the Central Plateau are able to produce more and larger young than females from Orford, strongly suggesting that reproductive output is not resource limited at the colder site, either by availability of food or by reduced opportunities for foraging due to the relatively colder environment.

4.4.4 Offspring size

Although the evolution of offspring size has received considerable attention in the life history literature (Smith and Fretwell, 1974; Brockelman, 1975; Parker and Begon, 1986; Lloyd, 1987; McGinley, Temme and Geber, 1987; Lalonde, 1991; Roff, 1992; Stearns, 1992), factors that influence offspring size and its variation in reptiles are not fully understood. In *N. ocellatus* there is considerable variability in offspring size between years (annual variation at both study sites) and between sites.

Annual variation within sites (within the average lifetime of individuals) is usually explained in terms of proximate effects such as resource availability and/or thermal differences, while interpopulational variation may be explained in terms of either evolved differences between populations (genetic differences) or proximate effects as a result

of climatic or other differences experienced by the populations. Despite RCM remaining relatively constant between years, the relationship between reproductive output and size of offspring differed between years. Year to year variation in mean offspring size may be interpreted as either adaptive (if optimum size varies between years) or as a consequence of mechanisms by which female lizards partition available energy into the clutch (Olsson and Shine, 1997). Variation in offspring size within each of the sites between years strongly suggests that offspring size in *N. ocellatus* is strongly influenced by proximate effects.

Although offspring characteristics differed between years at each site there were consistent differences among sites in offspring size, which may be interpreted as adaptive. Offspring from the Central Plateau were consistently larger (mass and length) than those from Orford. Life history theory suggests that fitness is highest in those individuals that produce the most offspring that survive to reproduce (Endler, 1986; Shine and Schwarzkopf, 1992; Sinervo and Doughty, 1996). A key determinant of clutch or litter size is therefore expected to be selection on size of neonates (Vitt and Price, 1982) and the amount of energy available to female lizards (Sinervo and Doughty, 1996). The evolution of offspring number and offspring size is presumed to be mediated by the trade-off between the fecundity advantage of producing small offspring and the advantages of producing larger offspring (Lloyd, 1987) that may have enhanced survival prospects (Ferguson and Fox, 1984; Forsman and Shine, 1995; but see Olsson, 1992; Sinervo *et al.*, 1992).

In all years offspring from the Central Plateau were significantly larger than those from Orford. In species occupying wide geographic ranges, larger offspring are often associated with colder climates, higher altitudes, or latitudes (e.g. Ferguson, Bohlen and Wooley; 1980; Berven and Gill, 1983; Forsman and Shine, 1995; Mathies and Andrews, 1995; Rohr, 1997) but this is not always the case (Sinervo, 1990b)). This response is thought to occur when potential for growth is low (Parker and Begon, 1986; Rowe, 1994; Forsman and Shine, 1995) or when there is strong selection on offspring survival (Brockelman, 1975; Rowe, 1994).

Further evidence for selection for larger offspring in cooler climates (or for later oviposition) is provided by the seasonal shift in egg size of many oviparous species that produce more than one clutch per season. Clutches produced later in the season are smaller, but individual egg size is larger (e.g. Nussbaum, 1981; Ferguson and Snell, 1986; DeMarco, 1989; Sinervo *et al.*, 1992; Sinervo and Doughty, 1996). This has been viewed as an adaptive response in parental investment per offspring to ameliorate some of the climatic disadvantages that late-born offspring face (Sinervo and Doughty, 1996). DeMarco (1989) predicted that females should increase offspring size at the expense of clutch size late in the season because larger offspring have a higher survival advantage in poor quality environments and because a larger body is a better buffer against poorer environmental conditions. Offspring size at birth is related to survival during the first month of life and its effects may persist to maturity (Sinervo *et al.*, 1992).

At the Central Plateau, offspring of *N. ocellatus* face three major challenges not faced by those from Orford which may select for larger offspring size at birth. They are born in an environment that is typically cooler than the Orford environment during any month (Chapter 2) which restricts their potential activity and feeding opportunities. Parturition also occurs approximately one month later (Chapter 3), which further restricts potential activity time prior to the winter period of inactivity. Finally, the winter at the Central Plateau potentially presents greater survival challenges to small lizards. A similar trend in offspring size has been identified in the small Australian skink, *Lampropholis delicata* (Forsman and Shine, 1995); they suggest that colder climates and shorter activity seasons may select for large offspring in southern populations because in these sites growth potential prior to winter is poor. The growth potential of offspring from Orford and the Central Plateau in different environments is further investigated experimentally in Chapter 6.

Multiple factors may have been involved in selecting for larger offspring size at the Central Plateau study site. In addition to those considered above, competition between juveniles may select for different sized

offspring (Parker and Begon, 1986). However, there appears to be little competition among juvenile *N. ocellatus* at either of the study sites, and no agonistic behaviour was observed between juveniles. Furthermore, juveniles captured during their first season had none of the bite marks that are generally considered to be evidence of interaction. Adults, however, often show extensive bite marks, on their legs, tails and torso (unpublished data).

Differences in offspring phenotype need not necessarily reflect evolved responses to selective pressures, such as those identified above. Alternatively, such differences may reflect phenotypically plastic responses to proximate environmental effects, such as maternal basking behaviour (see Chapter 7 and references therein) or increased resources available to females (e.g. James and Whitford, 1994; but see Ford and Seigel 1989a; Rohr, 1997). Factors affecting offspring phenotype are investigated further in Chapter 7.

4.4.5 Trade-off between clutch size and offspring size within populations

Assuming a finite limit to the energy available for reproduction, the investment in individual offspring is determined by the trade-off between the allocation of resources between offspring size and offspring number, and we can expect a negative relationship between these two variables (Brockelman, 1975; Ford and Seigel, 1989b; Schwarzkopf, 1992; Forsman and Shine, 1995).

In *N. ocellatus* there was a significant trade-off between offspring number and offspring size within each study population during 1996/1997. Large offspring result when an individual female produces relatively few young for her size. A negative relationship between size and number of offspring has been recorded in four of seven species of lizards reviewed by Ford and Seigel, (1989b) and more recently by Sinervo (1990b), Schwarzkopf (1992), Smith, Ballinger and Rose, (1995) and Olsson and Shine (1997).

A trade-off between number and size of offspring will result when energy available for reproduction is limited. For example, if total investment in a clutch is not some whole number multiple of the optimal egg size, production of either the next highest whole number of smaller than optimal eggs, or the lowest whole number of larger than optimal eggs is possible (Ebert, 1994; see also previous discussion on annual variation in offspring size). Olsson and Shine (1997) identify food availability as the likely limiting factor that induces a trade-off between offspring size and clutch size in natural populations, although weather conditions may also affect foraging ability (e.g. Avery, 1978; Avery, Bedford and Newcombe, 1982; Van Damme, Bauwens and Verheyen, 1991). I demonstrated a trade-off in offspring size and number in one year only (although it was demonstrated in both populations), thus clear conclusions to its cause are not possible. Several factors may restrict the amount of energy available for reproduction in a particular season. The amount of stored energy prior to vitellogenesis is likely to be especially important in *N. ocellatus* (see Chapter 3), and thus the trade-off between offspring size and number will be determined by energy stores established in the preceding year. The amount of stored energy is likely to be determined by a combination of food availability and foraging opportunity.

Chapter 5

Age and size relationships in *Niveoscincus ocellatus*

5.1 Introduction

Mature *Niveoscincus ocellatus* from the Central Plateau study site are significantly larger at maturity and reach a larger maximum size than those from the Orford study site (see Chapters 3 and 4). As was demonstrated, these size differences are largely responsible for the variation in key life history traits between the populations, particularly the high reproductive output (clutch mass and number of young) of females from the Central Plateau compared to females from Orford. In this chapter, I investigate the relationship between size and age of *N. ocellatus* and the possible reasons for differences in size at maturity and maximum size between the Central Plateau and Orford populations using the technique of skeletochronology. Although there has been a large number of intraspecific studies of variation in life histories of lizards, few have used skeletochronology for age determination.

Age at maturity is a pivotal trait because fitness is often more sensitive to changes in this trait than in any other (Stearns, 1992; Bernardo, 1993; Galán, 1996). Demographic pressures to mature early must be balanced by trade-offs with other fitness components to explain delayed maturity (Stearns, 1992). The advantages in delaying maturity and limiting reproduction include the opportunity for additional growth to larger body sizes. In species in which fecundity is closely related to female body size (which includes *N. ocellatus*), a delay in reproduction, although costly to current reproduction, may result in attainment of larger body size and greater gains in future fecundity (Stearns and Crandall, 1981; Bruce and Hairston, 1990; Stearns, 1992).

Species that delay maturity tend to be large and long-lived, whereas low survival rates in populations are often associated with early maturity (Stearns, 1992; Schwarzkopf, 1994). Any variation in size and age at maturity has important implications for the life history pattern of a particular population because after maturity energy is diverted away from growth, maintenance, and storage and directed towards reproduction (Andrews, 1982; Adolph and Porter, 1996; Rohr, 1997); in populations that mature at a smaller size, maximum size is also typically smaller (Galán, 1996; Sinervo, 1990a).

Longevity and age of attainment of sexual maturity vary widely among species of squamates (Heatwole and Pianka, 1993) and affect demographic strategies populations (Tinkle, Wilbur and Tilley, 1970; Dunham, Miles and Reznick, 1988). There are two general strategies of maturation in lizards: "early" versus "late" reproduction (Tinkle, Wilbur and Tilley, 1970), with natural selection favouring successful genotypes under certain environmental conditions (James, 1991b). Many authors have described a high variability in the size and age at maturity in female lizards, either between populations or within populations (e.g. Ballinger, 1979; Dunham 1982; Howland, 1992; Grant and Dunham, 1990, Tinkle, Dunham and Congdon, 1993; Rohr, 1997).

Differences between populations may result from several factors, including proximate factors acting on the population. Many studies address the role of temperature as a proximate source of variation in ectotherm life histories, including lizards (e.g. Conover and Present, 1990; Adolph and Porter 1993; Ferguson and Talent, 1993; Bernado, 1994; Adolph and Porter 1996). The thermal environment determines the growing season for many reptiles. Reptiles at low latitudes or low elevations may be active much of the year, whereas the growing season is curtailed at high elevations or high latitudes (Adolph and Porter, 1996). Furthermore, differences in daily thermal conditions during the active season also influence activity and are also likely to influence growth rate (Sinervo and Adolph, 1989; 1994 and Chapter 6 and references therein).

Recently, Adolph and Porter (1996) developed a model in which age and size at first reproduction may be predicted for lizards living in different thermal environments. In their model, lizards living in comparatively cold climates are predicted to delay maturity because of reduced opportunities for growth, and as a result mature at a larger sizes at an older age. Predictions from their model (Adolph and Porter, 1996) appear to be supported in the literature. Lizards inhabiting cold climates often exhibit delayed maturity and larger size at maturity (e.g. Tinkle, Wilbur and Tilley, 1970; Bruce and Hairston, 1990; Grant and Dunham, 1990; Galán, 1996; Rohr, 1997). However, other factors may be required to fully explain a delay in maturity (size or age). For example, Rohr (1997) compared two populations of *Eulamprus tympanum* separated by a steep altitudinal gradient and found that although the observed growth rates differed (those at high altitude predictably showed a slower growth rate), the alpine population delayed maturity more than was predicted by their respective growth rates and sizes.

Reptiles, like most vertebrates and invertebrates, begin reproducing before they reach their maximum body size (Galán, 1996) and in many reptiles, including skinks, sexual maturity is generally determined by minimum size, rather than age. Galán (1996) in a review of age to maturity in lizards found that most lizards mature in 1-2 years; however age to maturity is size dependent and larger species may take longer to reach maturity.

It is widely assumed that reptiles (and amphibians) show indeterminate growth (although growth slows after maturity (Andrews, 1982; Schwarzkopf, 1993; 1994)) and that body size and age are therefore positively correlated, although the relationship is usually quite weak and there is typically considerable variance in body size within a given age class. Consequently it is not justified to use data on body size as an accurate and reliable indicator of the age of an individual. In this chapter, the relationship between age and size is investigated using actual ages of individual lizards based on data obtained from skeletochronology.

There have been few attempts to examine differences in size and age at maturity in Australian skinks from different climates and particularly from cold temperate climates (but see Rohr, 1997). In this chapter I address the following questions:

1. are the differences in adult body size between the two populations a reflection of different growth rates that result from proximate environmental factors?
2. are the differences in maximum size a result of larger size at maturity at the Central Plateau or do they reflect enhanced longevity?
3. are there any observable intersexual differences in growth patterns at either study site?, and
4. do growth rates slow following sexual maturity?

5.2 Materials and methods

5.2.1 Skeletochronological techniques

There are several approaches that can be taken to establish age and size relationships in reptiles. In this study, the relationship between size and age was established using the technique of skeletochronology. The sexual maturity (or otherwise) of individuals was determined by examination of internal organs and comparison to known data on gonad development (Jones, Wapstra and Swain, 1997; Chapter 3) and minimum size at sexual maturity (Chapter 4 and unpublished data).

Skeletochronology has several advantages over other methods of estimating size and age relationships. Recapture of known individuals (capture-mark-recapture) and extrapolation from size-frequency data are very labour intensive and rely on recapturing a large proportion of lizards or capturing an unbiased cross section of the population (Halliday and Verrel, 1988). Capture-mark-recapture methods have the further disadvantage that species may be long-lived (e.g. in excess of 10 years for *Tiliqua rugosa* (Bull, 1994) and up to 12 years for *Eulamprus tympanum*

(Rohr, 1997)) and thus this method may take a long time to yield results. Estimations of age based on frequency distributions of size classes rely principally on unbiased sampling, which often proves difficult. Furthermore, it relies on little variation in growth rate within size classes which is not typical in reptiles (Andrews, 1982; Halliday and Verrell, 1988).

Skeletochronology is based on the observation that growth in amphibians and reptiles is periodic and its temporal pattern is recorded in certain bones. The general principle in ageing reptiles and amphibians by the use of bones lies in the use of Lines of Arrested Growth (LAG) or Resting Lines (RL) in bone, the number of which roughly corresponds to the individual age (Castanet and Smirina, 1990). There are a variety of terms in the literature for these lines; in this Chapter, the term LAGs will be used. Most of the lines present in skeletal tissues of many vertebrates are the result of arrested bone growth during hibernation (cold) or aestivation (drought) periods (LeClair and Castenet, 1987). The growth and histology of reptile bones is well described by Romer (1956), Enlow (1969) and Ricqlès (1976) and it is established that long bones (such as the femur or phalanges) increase in diameter throughout the life of the individual through the deposition of bone at the outer edge or periosteum (e.g. Romer, 1956; Enlow, 1969 (Figure 5.1)). The rate of deposition varies both seasonally (producing denser bone during periods of slow growth which correspond to the LAGs when stained histologically) and during the lifetime of an individual.

Although skeletochronolgy is a reliable method for estimating the age of an individual, there are several difficulties that must be overcome. The major difficulty involves the loss of LAGs laid down early in life. The size of the medullary cavity often increases during this development through resorption and redeposition of bone within the medullary cavity (Enlow, 1969). This resorption must be taken into account in estimating the age of individuals (see Hemelaar, 1985; Castanet and Smirina, 1990), and may result in partial or complete loss of early LAGs and could result in an underestimation of an individual's age. This resorption is lowest

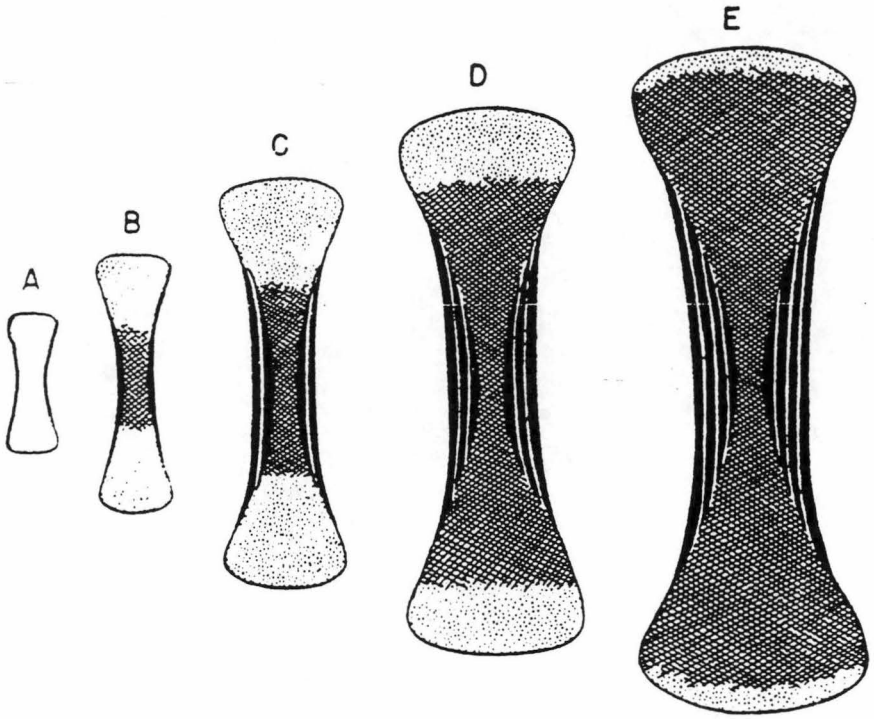

Figure 5.1. Development of a long bone in a reptile (taken from Romer, 1956). *A* represents the bone immediately after embryonic development, *B-E* represents the growth of the bone during the life of the individual. Black lines represent new perichondral bone laid down around the outer surface. The interior medullary cavity is modified by resorption and redeposition and may in some cases obscure perichondral bone laid down early in life.

in the mid region of the diaphysis of the bone and the use of small individuals in which no LAGs have been lost can be used to back-calculate the number of LAGs in older animals (e.g. Hemelaar, 1985; LeClair and Castanet, 1987). Whatever the species and the population studied, the amount of bone resorption at the periphery of the medullar cavity is rarely extensive (Caetano, 1990). Resorption rates differ between populations, with high altitude populations sometimes displaying greater resorption (Estaban, 1990), presumably due, in part, to their greater age. Although the degree of endosteal resorption is related to age, there appears to be great variability in this phenomenon, with some old individuals showing all resting lines (Estaban, 1990; Haemlaar, 1985).

Almost any long bones are suitable for ageing; however, the femur is usually used. After preparation and sectioning, the bones are usually stained with haematoxylin. Cross-sections then reveal alternating dark and light concentric rings. The thin dark rings (LAGs) represent periods when little or no growth occurred. Between the resting lines are broad areas of lightly stained bone which represent periods of rapid growth and bone deposition. In studies of age and growth it is important to calibrate results (Frazier, 1985). A full year's growth typically consists of a broad band and its adjacent narrow resting line. The annual nature of these lines has been established in a number of studies (e.g. Smirina, 1972; Francillon, 1979; Pilorge and Castanet, 1981; Lykens and Forester, 1987; see Castanet *et al.*, 1993 for recent review). Although LAGs are most obvious in individuals living in high latitudes/altitudes (areas with marked seasonality) (Castanet and Smirina, 1990; Estaban, 1990; Castanet and Baez, 1991), their occurrence has also been reported in the bones of individuals living in aseasonal environments (Chinsamy *et al.*, 1995). It has been suggested that they result from endogenous biological rhythms that are synchronised by external factors.

In addition to ageing individuals using this method, it may also be possible to infer certain events through the lifetime of an individual. For example, it is often possible to infer the age when reproduction first took place because energy diverted to reproduction usually results in lower growth and the LAGs appear closer together than during phases of rapid

growth (Augert and Jolly, 1993). Although this method of ageing has now been available for some time the majority of studies have concentrated on individual age and sometimes on interindividual and intersexual polymorphism, but only within a single population. The study of interpopulational, as well as interspecific differences greatly increases the interest in this method (Castanet and Smirina, 1990). For example, Haemlar (1985; 1988) compared populations from different altitudes and latitudes and found distinct differences in growth rate and longevity.

5.2.2 Ageing of *Niveoscincus ocellatus*

There are a variety of specific methods available to obtain stained sections of long bones, and most researchers adapt general methods to specific success (e.g. Lykens and Forester, 1987; LeClair and Castanet, 1987; Hudson, 1988; Castanet, Newman and Saint Girons, 1988; Flageole and LeClair, 1992. All methods rely on decalcifying the bones (generally with 3% or 5% nitric acid for between 3 and 48 hours depending on the size of the bone) and staining with an aluminium or iron haematoxylin (e.g. Erlich's haematoxylin). In this study, femurs were dissected from lizards killed for other life history data. Femurs were then processed according to the following methodology.

Femurs were initially cleaned of surrounding tissue and stored in 70 % ethanol until processing. They were decalcified by placing in 5% nitric acid for 5-6 hours. Decalcification was halted by placing bones in tapwater for 24 hours. After this time bones were adequately softened and were embedded in paraffin wax using standard techniques after alcohol dehydration. Embedded femurs were serially sectioned at 10-12 μm and sections transferred to microscope slides.

Several staining regimes were tried initially and the best results were obtained after staining with Ehrlichs haematoxylin for 15-20 minutes followed by two rinses in tapwater for 3 minutes. Stained slides were then mounted and allowed to dry before examination. Slides were examined at 100x and 400x magnification. In femurs, the area of least

remodelling appears to lie toward the proximal end of the bone, rather than in the middle of the diaphysis. A blood vessel canal runs obliquely through the bone, which shows up as a sizeable hole in the cortex, and moves from the medullary cavity to the periphery of the section toward the distal end of the bone. The area of least remodelling occurred around this canal; distally the endosteal bone expands quite rapidly and can obscure much of the section (Hudson, pers. comm.). At this point, the outer and inner diameters were measured using an eyepiece graticule.

The number of LAGs was counted and the diameter of the first four in each individual measured (in order to provide an estimation of the number of LAGs lost due to resorption). In all cases, several sections were compared in order to establish that the number of LAGs was consistent (see Castanet and Smirina, 1990). There was little difficulty in locating and counting LAGs in the majority of specimens. However, in some cases, the correct part of the bone could not be located (due to difficulties during the histological process) or the staining was too dark or light to clearly define the LAGs. In these cases, age estimations of these individuals were not made. LAGs formed early in life are generally stained lighter (Hudson, pers. comm.), and that was the case in this study also. All bones were examined initially over a three day period, and then again in a blind retrial three weeks later to confirm the reliability of early estimations.

In *N. ocellatus* growth slows after sexual maturity and the distance between LAGs diminishes towards the periphery. In older individuals the number of LAGs at the periphery became more difficult to determine; in these cases, Castanet, Newman and Saint Girons (1988) suggest that a conservative estimate be made, which may result in the underestimation of the age of some older individuals.

5.2.3 Analyses of growth curves

The use of growth curves for reptiles has received considerable attention (e.g. Dunham, 1978; Schoener and Schoener, 1978; Andrews, 1982; James,

1991b; Sexton, Andrews and Bramble, 1992; Adolph and Porter, 1996; Niewiarowski *et al.*, 1997). In this study, the relationship between SVL and age was used to construct growth curves. Two asymptotic growth models based on linear length measurements are most often used in reptile studies, the von Bertalanffy and logistic-by-length models (Dunham, 1978, Schoener and Schoener, 1978; Andrews, 1982; Haemlar, 1988; James, 1991b; Sexton, Andrews and Bramble, 1992). The two models make different predictions about the shapes of the growth curves. The von Bertalanffy curve predicts that growth (in length) is maximum in the smallest lizards and slows as size increases, whereas the logistic- by-length model predicts maximal growth (in length) at 63 % of the asymptotic length (Andrews, 1982). *Niveoscincus ocellatus* grows most rapidly early in life (Chapter 6 and unpublished data); consequently, the von Bertalanffy growth model is fitted to the data. The von Bertalanffy model was the most commonly encountered in the literature on reptiles and is considered particularly appropriate in species that are long-lived (Andrews, 1982).

In this study, the von Bertalanffy model was used to compare growth of males and females at each site, and growth patterns between sites. The general form of the von Bertalanffy equation used is:

$$SVL_t = a\,(1\text{-}be^{-kt})$$

where SVL_t is the body size at time t, a is the asymptotic body size, b is a parameter related to initial body size, and k is the characteristic growth rate. The parameters a, b and k were estimated using nonlinear regression techniques (Andrews, 1982, James, 1991b) using SYSTAT for Macintosh (Wilkinson, 1989). The von Bertalanffy model provided an excellent fit to the data from the ageing of individuals (adjusted r^2 of 0.902 - 0.952). Growth trajectories were considered significantly different if the 95 % confidence planes did not overlap (Dunham, 1978; Schoener and Schoener, 1978; James, 1991b; Niewiarowski *et al.*, 1997). This is considered to be a conservative test for differences in growth rates (Schoener and Schoener, 1978; Henle, 1989).

Initially, intersexual differences at each site were investigated. Data from juveniles were included in data for both males and females to establish the best regression permitted for the correct evaluation of the age of animals in their early years (see Ryser, 1988). Juvenile male and female *N. ocellatus* do not show substantially different growth patterns in field enclosures (Chapter 6).

5.3 Results

5.3.1 Age and size relationships

Niveoscincus ocellatus were successfully aged using skeletochronology. In the majority of cases, distinct dark bands (LAGs) were evident in the growth zone of the bone, and these were assigned as periods of little or no growth during winter. The diameter of the first and second LAGs in juvenile and subadult animals were used for comparison to older animals to account for endosteal resorption. The advantage of this system was that the actual age (to the nearest month) of juvenile and subadult lizards could also be judged by their size class and their birth date. In most cases (but particularly in Central Plateau individuals) and in all adults the first LAG was lost, and in older animals the second LAG was also sometimes resorbed. In very old animals, the third LAG was partially resorbed, but in no cases was it impossible to assign an accurate age based on the size of this third partial ring. Thus, the age of an individual (in months) was calculated based on the number of winters it had lived through (ie. number of LAGS, including those calculated to have been lost by resorption), the date of birth (assumed to be January for Orford and February for the Central Plateau) and the date at which it was captured. LAGs generally stained very well, but in some cases staining was too light for accurate resolution, and data from these individuals were discarded.

As in a number of previous studies (e.g. Caetano, 1990; Estaban, 1990; Francillon-Viellot, Arntzen and Géraudie, 1990), the distance between

LAGs decreases rapidly toward the periphery in *N. ocellatus* and is particularly evident after sexual maturity.

Figure 5.2 presents the relationship between size and age at the Orford study site. Clearly, early in life, growth is rapid, and there is a strong relationship between size and age. Sexual maturity at the Orford population is reached at an approximate size of 55-57 mm, and this size is reached towards the end of an individual's second year. Males and females may begin reproductive activity (late summer, male gonadal development; autumn, mating and vitellogenesis in females) at the end of their second season, and females produce their first clutch in their third season. After maturity, growth slows considerably, and the relationship between age and size is not as strong. The oldest females were 96 months (8 years) old, and the oldest male was 72 months old (6 years). From the individuals aged, females appear to have the highest longevity, although this may be a result of sampling biases (more larger females were sampled).

The largest difference in the relationship between size and age at the Central Plateau and Orford is the delay in maturity exhibited by individuals from the Central Plateau (Figure 5.3). Growth does not permit individuals to reach the same size as Orford individuals by the end of their second season, and at this time they are between 48 and 54 mm. Consequently, maturity is delayed until the end of their third season, and as a result individuals at the Central Plateau mature at a larger size than those from Orford. Maturity occurs at an approximate SVL of 65 mm (see also Chapter 4). Males were reproductively active at the end of their third season, and females were also beginning to mature. Consequently, the youngest females that produced a clutch were in their fourth season. Longevity at the Central Plateau is greater than at Orford; the oldest females were 120 (10 years) and 132 (11 years) months old, and the oldest males were 132 (11 years) and 144 (12 years) months old.

5.3.2 Growth curves

Table 5.1 shows the parameter values and the upper and lower 95 % confidence intervals estimated from the model ($SVL_t = a\ (1\text{-}be^{-kt})$); the parameters are as described above. Figures 5.4 and 5.5 present the von Bertalanffy growth curves for male and female *N. ocellatus* at Orford and the Central Plateau respectively constructed from these values. The data provide an excellent fit to the data in all groups ($r^2 = 0.91$ - 0.96). Clearly, within each site there is considerable overlap of the growth curves of males and females, indicating that the sexes do not show markedly different growth patterns.

Sexes from each site were consequently pooled to compare von Bertalanffy growth curves between Orford and the Central Plateau. The parameter values and upper and lower 95 % confidence intervals for the von Bertalanffy growth curves based on the combined data are shown in Table 5.2.

Table 5.1. Parameter values for von Bertalanffy growth model generated from nonlinear regressions of size (SVL) and age for male and female *Niveoscincus ocellatus* at Orford and the Central Plateau. Upper and lower values are the 95 % confidence intervals.

	Parameters from von Bertalanffy model									
	a			b			k			r^2
group	lower	mean	upper	lower	mean	upper	lower	mean	upper	
Orford males	62.65	**67.01**	71.38	0.51	**0.55**	0.59	0.029	**0.042**	0.054	0.95
Orford females	64.57	**66.99**	69.41	0.52	**0.55**	0.58	0.034	**0.043**	0.052	0.96
CP males	76.51	**79.55**	82.59	0.58	**0.63**	0.67	0.022	**0.027**	0.032	0.91
CP females	81.72	**85.33**	88.94	0.62	**0.65**	0.69	0.018	**0.022**	0.026	0.94

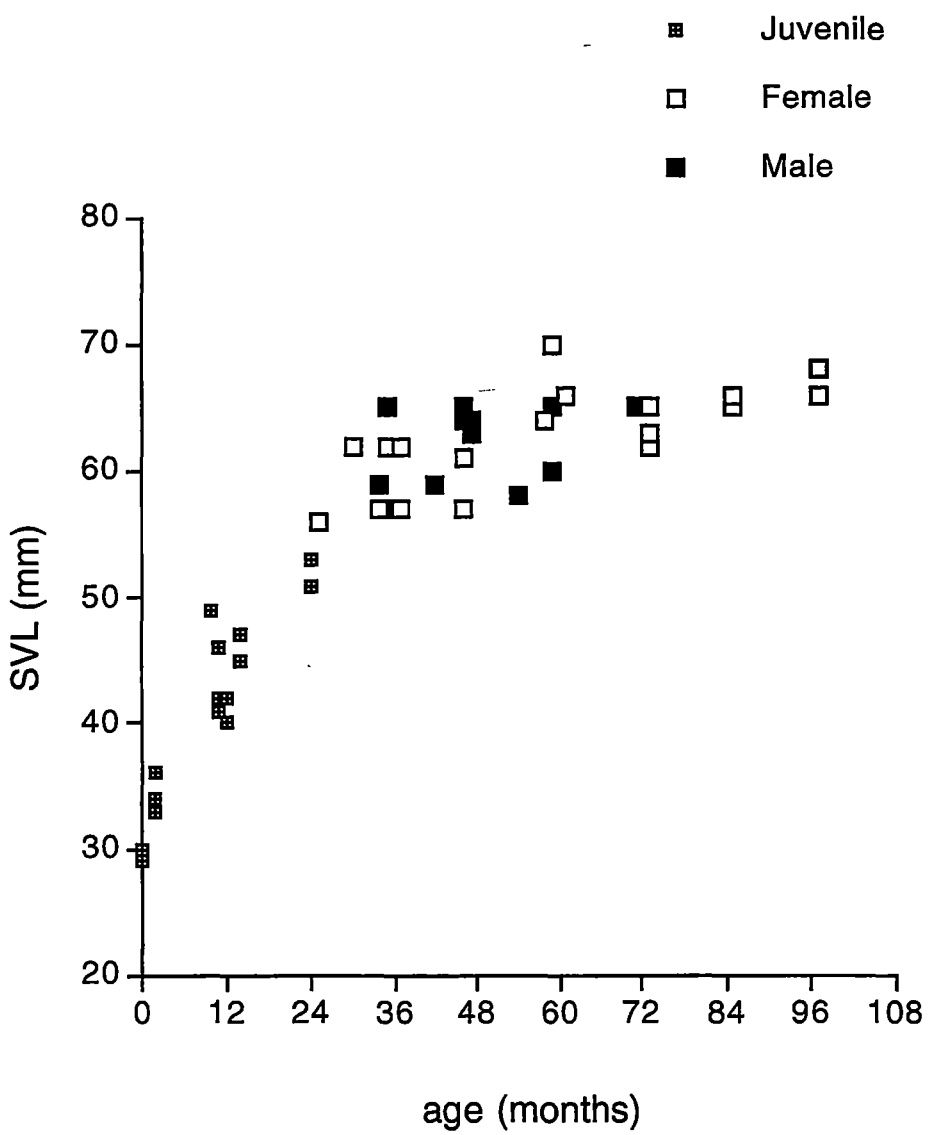

Figure 5.2. Relationship between age and size in *Niveoscincus ocellatus* at the Orford population. Age (in months) is estimated from the number of LAGs, the capture date and the assumption that all individuals were born in January (Chapter 3).

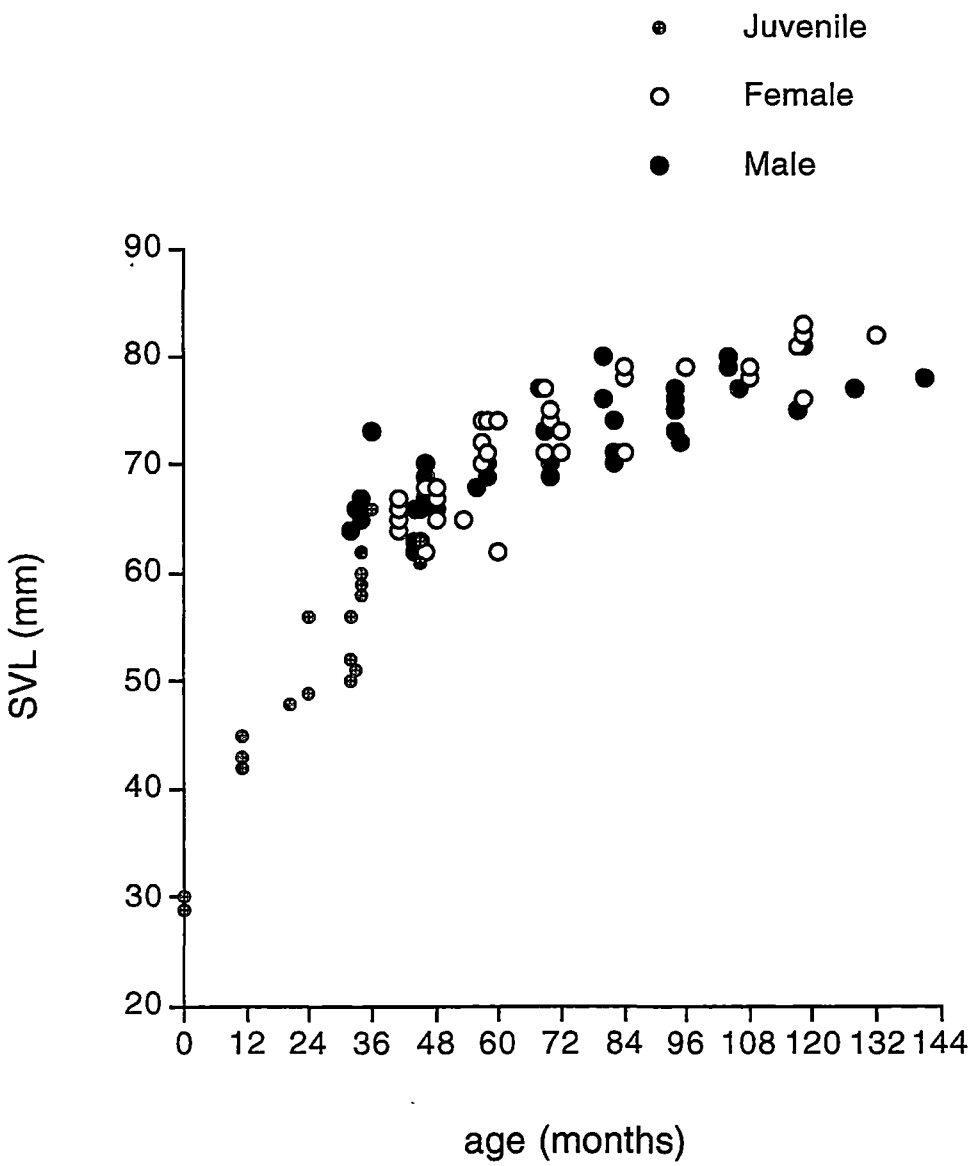

Figure 5.3. Relationship between age and size in *Niveoscincus ocellatus* at the Central Plateau population. Age (in months) is estimated from the number of LAGs, the capture date and the assumption that all individuals were born in February.

Table 5.2. Parameter values for von Bertalanffy growth model generated from nonlinear regressions of size (SVL) and age for *Niveoscincus ocellatus* at Orford and the Central Plateau. Upper and lower values are the 95 % confidence intervals.

	Parameters from von Bertalanffy model									
	a			b			k			r^2
population	lower	mean	upper	lower	mean	upper	lower	mean	upper	
Orford	64.49	**66.66**	68.82	0.52	**0.55**	0.58	0.036	**0.044**	0.053	0.95
Central Plateau	78.81	**81.08**	83.35	0.60	**0.63**	0.67	0.023	**0.026**	0.030	0.90

Figure 5.6 presents the von Bertalanffy growth curves constructed from these parameters. These curves are an excellent fit for the data (adjusted r^2: Orford, 0.95; Central Plateau, 0.90). Clearly, the growth patterns exhibited at the two sites differ significantly. There are few differences in growth rate early in life, but the growth curves diverge substantially at two years of age. This is consistent with the observed ages at maturity. Orford individuals are large enough to be mature at two years of age (females produce their first clutch in their third season) and as a result growth slows. At the Central Plateau site, maturity is reached on average one year later (females generally produce their first clutch in their fourth season) and growth slows at approximately this point. The asymptotic growth points of the two populations differ markedly. The asymptote for the Orford population is between 64 and 69 mm, and for the Central Plateau population it lies between 79 and 83 mm. Both of these values are in close agreement with the largest individuals caught from the populations during the course of this study.

The lower value of k (growth coefficient) at the Central Plateau does not necessarily imply an absolute lower growth than at Orford, but is a reflection of the time taken to reach their growth asymptote. Thus, the lower value of k at the Central Plateau is a result of the delay in maturity at this site, and the consequent longer period of rapid growth prior to maturation.

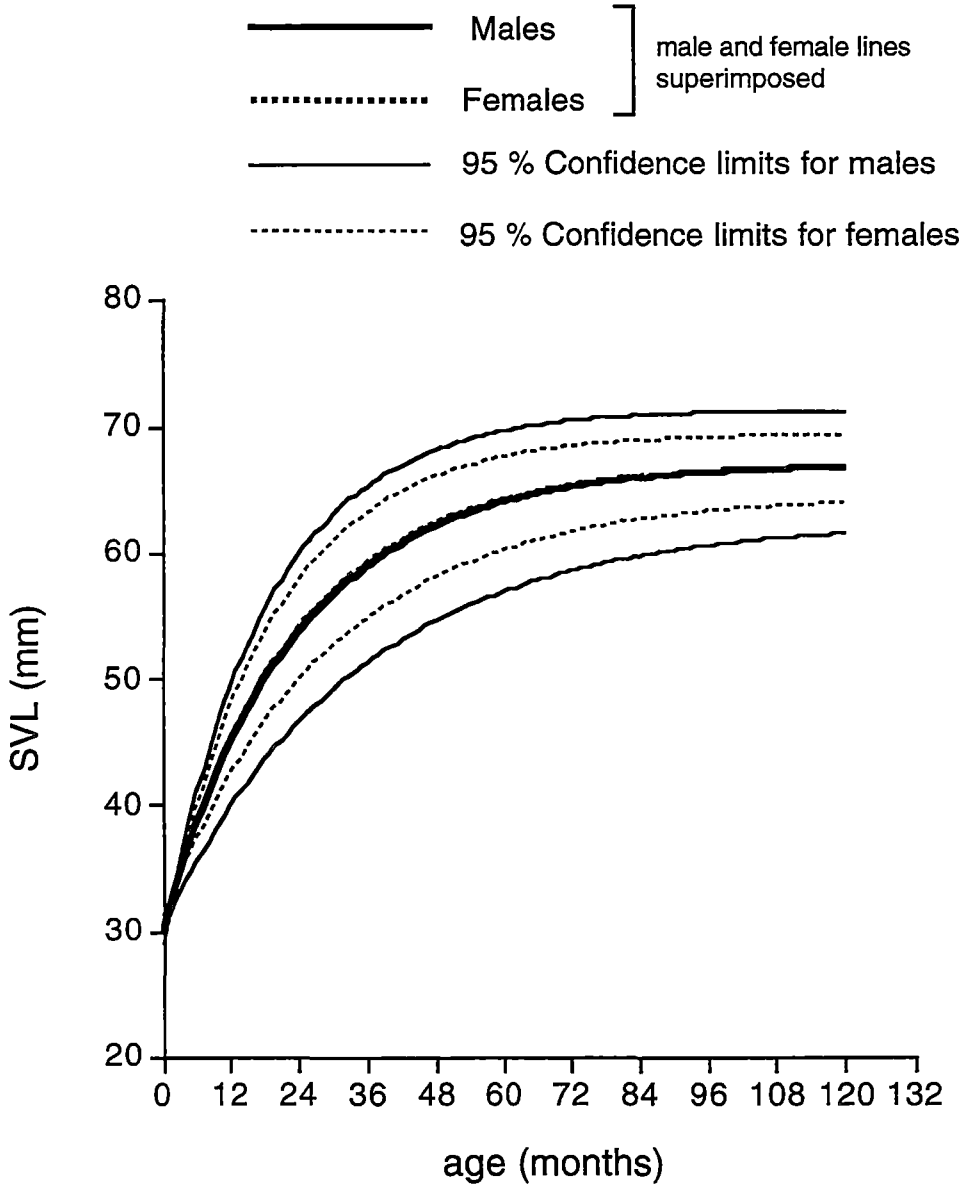

Figure 5.4. von Bertalanffy growth curve based on data from male and female *Niveoscincus ocellatus* from Orford. Juvenile data are included in the construction of curves for both sexes. Equations for these curves are found in Table 5.1.

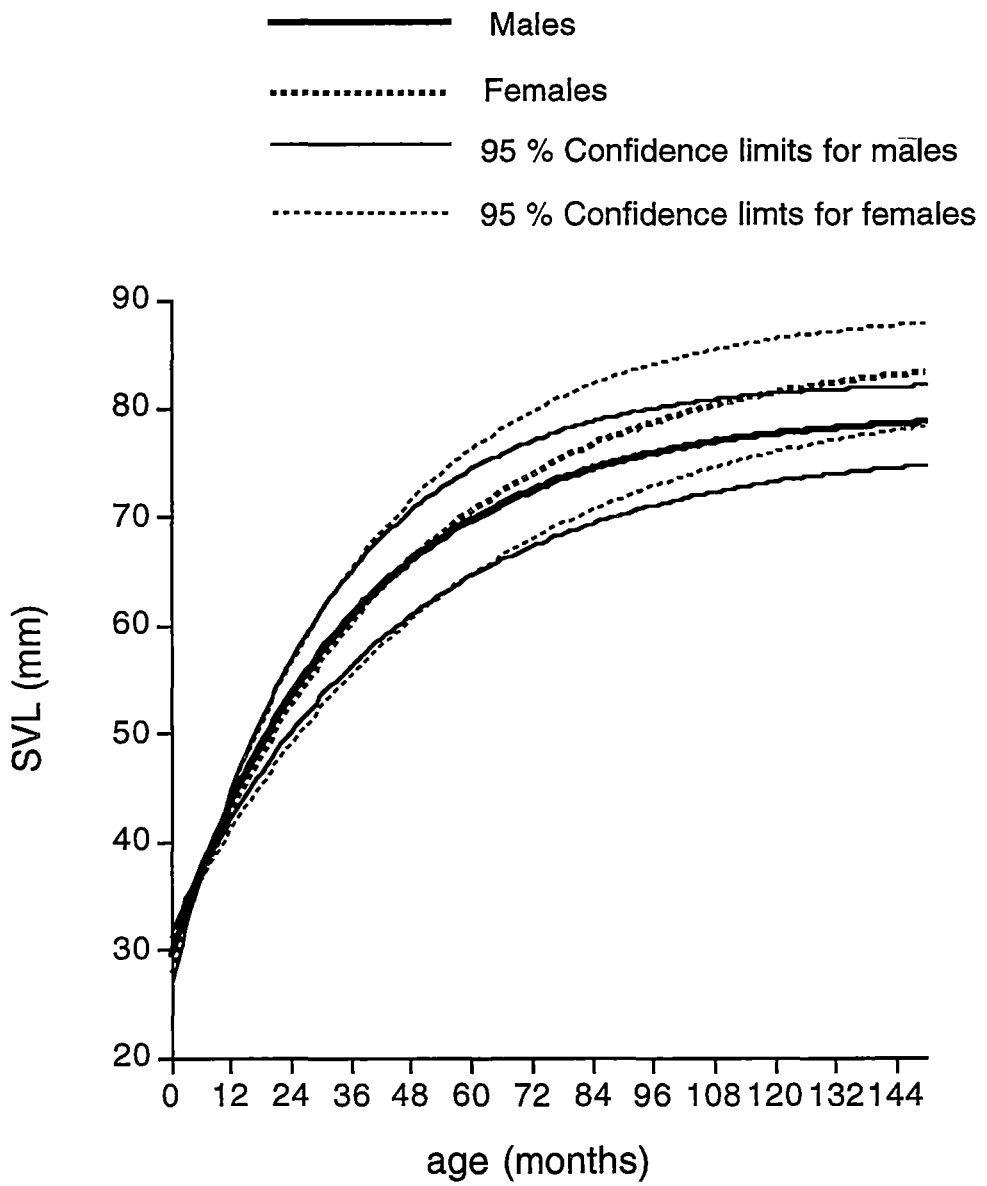

Figure 5.5. von Bertalanffy growth curve based on data from male and female *Niveoscincus ocellatus* from the Central Plateau. Juvenile data are included in the construction of curves for both sexes. Equations for these curves are found in Table 5.1.

5.4 Discussion

Skeletochronology provides a suitable and reliable method by which to establish the age of *Niveoscincus ocellatus*. In both populations, distinct dark bands were formed during the period of winter inactivity and the only difficulty encountered was the loss of early LAGs due to expansion of the medullary cavity and faint staining of early lines, especially in the Central Plateau individuals. However, these problems were overcome through the measurement of early lines and bone diameters in juvenile lizards.

There are no intersexual differences in the relationship between size and age or in the von Bertalanffy growth curves at either site. This is consistent with the observations that juvenile *N. ocellatus* growth rates do not differ between sexes (Chapter 6) and that there are no intersexual differences in the minimum size at maturity or maximum size at either site. Growth in both populations is rapid prior to maturation, but slows markedly after this point. This is the typical pattern of growth in reptiles; the slowing of growth after maturation is associated with the energy demands of gonadal maturation and other costs associated with reproduction (Andrews, 1982; Anderson and Vitt, 1990; Shine and Schwarzkopf, 1992; Bernardo, 1993; Schwarzkopf, 1993; 1994).

The fact that males and females mature at a similar size in both populations and growth slows after maturation in both sexes implies that sexual maturity is equally energetically costly to males and females (Anderson and Vitt, 1990). While the energetic costs associated with female reproduction are well documented (see later Chapters 3, 7,8 and references therein), there is less information on the energetic costs for male reproduction. Male *N. ocellatus* undertake annual gonad development each summer which deplete energy reserves (Chapter 3).

Skinks may be expected, due to their relatively small size, to mature relatively young and indeed this is found in many small species which mature within one year (*Morethia boulengeri* (Henle, 1989);

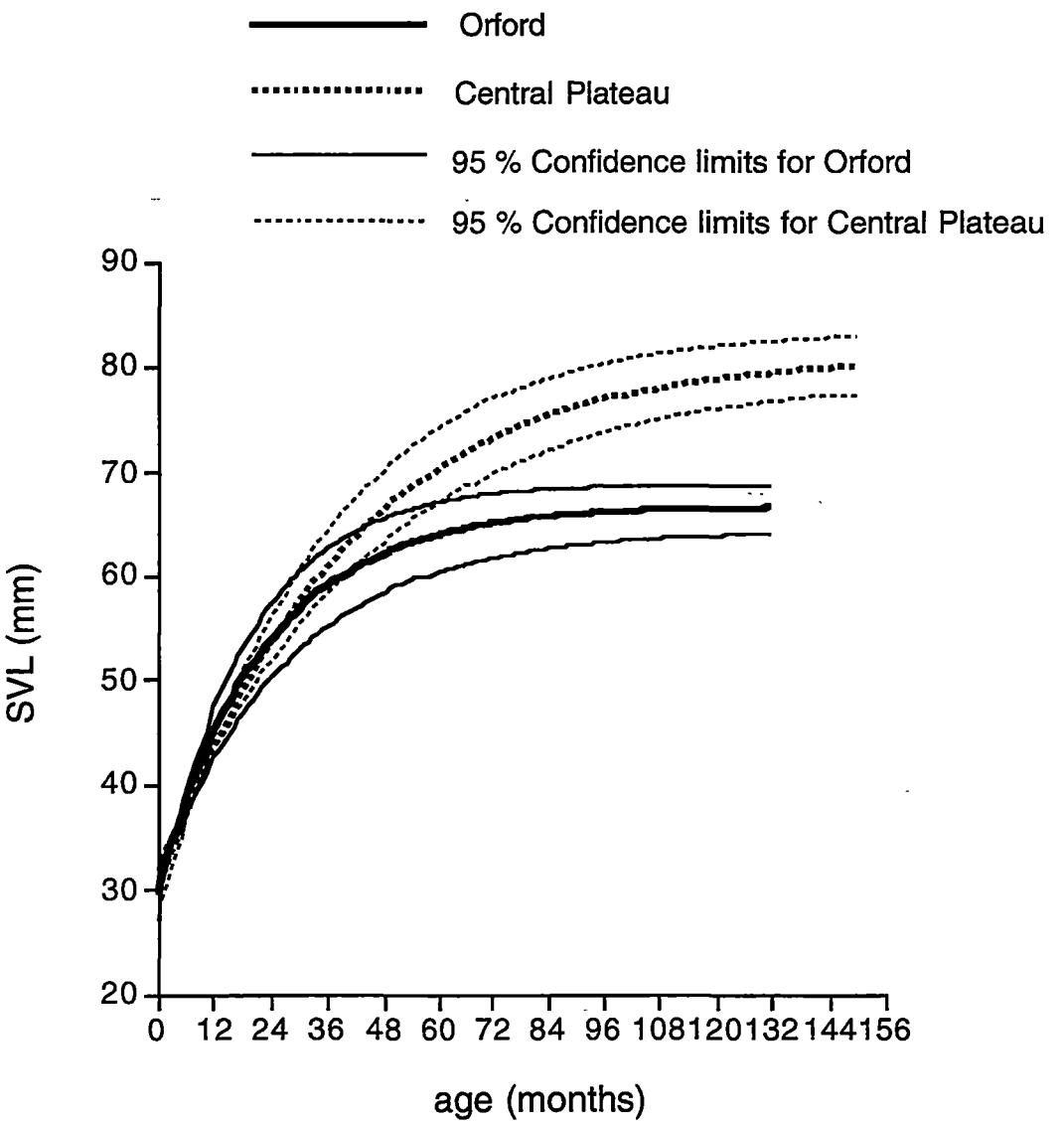

Figure 5.6. Von Bertalanffy growth curve based on data from *Niveoscincus ocellatus* from Orford and the Central Plateau. Juvenile data are included in the construction of curves for both populations. Equations for these curves are presented in Table 5.2.

Lampropholis delicata and *L. guichenoti* (Hudson, 1988), but other small to medium sized species mature more slowly (*Bassiana duperryi* and *Pseudemoia pagenstecheri* (Hudson, 1988) and *Niveoscincus* species (Hudson, 1997), and larger skinks such as *Tiliqua rugosa* are longer lived and may not reach maturity for five years (Bull, 1994). Greer (1989) provides a further summary of the estimated age to maturity in Australian skinks.

The data from the present study are in close agreement with the data of ages at maturity and average ages of mature *N. ocellatus* estimated by Hudson (1997). Hudson (1997) found that the mean age of mature female *N. ocellatus* (SVL ranged from 61-72 mm which is similar to the size range at the Orford study site) was 2-11 years, with females maturing late in their second or third year. Other populations of lowland *Niveoscincus* species (*N. pretiosus*, *N. metallicus*, and *N. coventryi*) have a similar age at maturity, while alpine species (*N. greeni* and *N. microlepidotus*) appear to mature at a later age (3-4 years) (Hudson, 1997).

There have been few studies that have examined differences in size and age at maturity in lizards occupying wide geographic ranges using known ages based on skeletochronological techniques, and even fewer of Australian skinks. Like many other life history traits, age and size at maturity differ between populations of *N. ocellatus*. Maturity is reached at the end of the second season at Orford, and at the end of the third season at the Central Plateau. The ages at maturity at the Central Plateau are more typical of the ages at maturity of the alpine species of *Niveoscincus* (Hudson, 1997).

Although age at maturity is an important life history parameter (Roff, 1992; Stearns, 1992; Bernardo, 1993; Rohr, 1997) relatively little attention has been paid to the evolution of age and size and maturity among populations experiencing different thermal environments (Adolph and Porter, 1996). Thermal effects on growth can greatly alter life histories (Adolph and Porter, 1996). Delayed maturity is common in lizards inhabiting colder climates (e.g. Tinkle, Wilbur, and Tilley 1970; Dunham, Miles and Reznick, 1988; Adolph and Porter, 1996; Rohr, 1997) and is

often associated with an inability of lizards occupying these habitats to reach a size suitable for reproduction at a time suitable for reproduction at the same age as those from warmer climates (Andrews, 1982; Jones, Ballinger and Porter, 1987; Adolph and Porter, 1996). For example, in populations of *Sceloporus undulatus*, rapid growth of one population is associated with relatively early maturity, small adult body size, and high adult mortality rates (Niewiarowski, 1995), while slow growth in another population is associated with relatively delayed maturity, large adult body size and low adult mortality rates (Jones and Ballinger, 1987; Niewiarowski, 1995). This appears to be the case in this study; *N. ocellatus* from the Central Plateau have reduced growth potential because they are born later in their first season and the thermal characteristics at the site are less favourable for growth (see Chapter 6). As a result, while individuals from Orford are able to reach the minimum size for reproduction within two years (SVL ~ 55 mm), individuals from the Central Plateau cannot do so.

The result of the delay in maturity is that lizards from the Central Plateau are able to devote available energy to growth (rather than reproduction) for one more year than lizards from Orford. Consequently, lizards at the Central Plateau mature at a larger size than those from Orford. This is a common pattern observed in geographically widespread species (e.g. Ballinger and Congdon, 1981; Grant and Dunham, 1990; Landwer, 1990; Shine and Charnov, 1992; Ferguson, Snell and Landwer, 1993; Rohr, 1997). However, an alternative strategy by lizards from populations occupying cold habitats is to mature at the same age as their counterparts from milder habitats, but at smaller sizes (e.g. Parker and Pianka, 1975; Forsman and Shine, 1995; Mathies and Andrews, 1995). For example, Mathies and Andrews (1995) found that high elevation reproductive female *Sceloporus scalaris* were smaller than low elevation females and Forsman and Shine (1995) found that southern populations of *Lampropholis delicata* were smaller than populations from northern, warmer populations.

Delaying maturity can be very costly to individual fitness, prompting Tinkle and Dunham (1986) to conclude that strong trade-offs must exist

between fitness losses due to delayed reproduction and gains attendant on the delay. In stable populations, age at maturity is thought to evolve so as to maximise lifetime reproductive success (Bernardo, 1993; Adolph and Porter, 1996); the strategies adopted by *N. ocellatus* at each of the study site with respect to age and size at maturity support this idea. At Orford, adult survival is comparatively low and growth comparatively rapid and lifetime fecundity is maximised by rapid maturation. Conversely, at the Central Plateau survival of adults is relatively high (maximum age 12 years), growth is relative slow, and lifetime fecundity is maximised by delaying maturity and devoting available energy to growth. As a result, fecundity in subsequent years is enhanced (Shine, 1978; Madsen, 1987; Shine and Schwarzkopf, 1992). In this case, delaying reproduction by one year results in a small potential fecundity cost in that year, but this is made up for in subsequent years by a high reproductive output (Stearns and Crandall, 1981; Bruce and Hairston, 1990; Stearns, 1992). Furthermore, age at first reproduction may be further delayed at high altitude or in cold climates because resources are limited and lizards are unable to devote energy to both growth and reproduction (Rohr, 1997). Larger lizards have a greater capacity to store resources (Schwarzkopf, 1992; 1994; DeMarco, 1989) and may be able to reproduce when smaller lizards are unable to do so.

Differences in growth rate are often used to explain differences in life history traits, including size and/or age at maturity (e.g. Halliday and Verrell, 1988; Grant and Dunham, 1990; Bernado, 1993; Ferguson and Talent, 1993; Mateo and Castanet, 1994; Smith, Ballinger and Nietfeldt, 1994; Adolph and Porter, 1996). In my study, the different growth patterns exhibited by *N. ocellatus* at the two study sites result in differences in adult body size that in turn affect other life history traits. This study supports the recent view (Ferguson, Landwer and Snell, 1990; Ferguson and Talent, 1993; Adolph and Porter, 1996; Rohr, 1997) that age and size at maturity are phenotypically plastic traits modified by the thermal environment. In Chapter 6 I further investigate factors that influence growth rate, particularly whether there are any underlying genetic differences between populations.

Chapter 6

The influence of the thermal environment on growth rate in juvenile *Niveoscincus ocellatus*

6.1 Introduction

Life history characteristics vary widely among species of lizards and between populations of species (Tinkle, Wilbur and Tilley, 1970; Dunham, Miles and Reznick, 1988) and species with broad distributions often display a large amount of geographic variation in life history traits. Geographic variation in life history traits has received both theoretical and empirical attention, with the aims of testing hypotheses dealing with life history evolution, and of investigating possible sources of variation (reviewed in Roff, 1992 and Stearns, 1992).

Most authors seek adaptive or phylogenetic explanations for life history patterns, since these are usually presumed to reflect genetic differences (Ferguson and Talent, 1993). However, lizard life histories are often phenotypically plastic, responding to temperature, food availability and other environmental factors. Consequently, distinguishing between phenotypic plasticity induced by proximate environmental influences and genetic divergence is fundamental to understanding the ecological and evolutionary significance of intraspecific geographic variation (Niewiarowski and Roosenburg, 1993; Sorci, Clobert and Belichon, 1996). Studies of intraspecific variation are typically concerned with identifying environmental factors that are correlated with observed life history variation.

Many reptilian life history traits are phenotypically plastic (e.g. Ford and Seigel, 1989a; Seigel and Ford, 1991; Niewiarowski and Roosenburg, 1993; Rohr, 1997); they exhibit a norm of reactions across different environments rather than expressing the same phenotype (e.g. Stearns,

1989a; Sinervo and Adolph, 1994). Phenotypic plasticity of many physiological and life history traits represents "phenotypic modulation" (Smith-Gill, 1983), in which traits respond passively to environmental variables such as temperature, salinity, and food abundance and reflect chemical and physical effects on underlying biochemical and developmental processes (Sinervo and Adolph, 1994).

Growth rates in particular are often highly variable (Stearns and Koella, 1986) and are probably the most widely studied phenotypically plastic trait. Growth rate in squamates varies interspecifically, intraspecifically between the sexes, and with age, nutritional state and level of stored energy (Andrews, 1982; Heatwole and Pianka, 1993). It is usually rapid in hatchlings or newborns, declines towards sexual maturity and thereafter is even slower as maximum size is approached.

In reptiles, growth rates are affected by a number of environmental factors, particularly food availability and thermal environment (Ballinger, 1977; 1983; Dunham, 1978; Ballinger and Congdon, 1980; Stamps and Tanaka, 1981; Andrews, 1982; Barnett and Schwaner, 1985; Christian, 1986; Sinervo and Adolph, 1989; 1994; Sinervo, 1990a; Niewiarowski and Roosenburg 1993; Smith and Ballinger, 1994). Recently, Adolph and Porter (1993) developed a model describing the proximate effects of temperature on lizard life history traits. They were able to show that patterns of life history variation may be explained or predicted without invoking any underlying genetic basis, thus emphasising the "tremendous potential role" played by proximate factors in determining lizard life histories.

Recently there has also been a series of studies in which the sources of variation in life history traits (especially growth rate) has been investigated using reciprocal transplant experiments (e.g. Bernado, 1993; Niewiarowski and Roosenburg, 1993) or common garden experiments (e.g. Ferguson and Brockman, 1980; Sinervo and Adolph, 1989; Sinervo, 1990a; Ferguson and Talent, 1993; Smith, Ballinger and Nietfeldt; Schwarzkopf, 1996b; Sorci, Clobert and Belichon, 1996). Although these studies have identified large proximate environmental effects leading to

variation in phenotype, they have also often shown that there is an underlying genetic difference between populations. If a genetic basis for variation in life history traits exists within populations, then this facilitates adaptation to local environmental conditions (Sorci, Clobert and Belichon, 1996).

Explaining geographic variation in growth rates poses a particularly significant challenge for those studying life history evolution, because variation in growth rate has direct consequences for many other life history traits (Sinervo, 1990a; Bernado, 1993; Niewiarowski, 1995). Growth is a central life history trait through its influence on adult body size, fecundity, and age and size at maturation (Sinervo and Adolph, 1989; Ferguson and Talent, 1993; Niewiarowski, 1995; Roosenburg and Kelley, 1996). Environmental effects on this trait are significant for two reasons: (1) they confound our ability to analyse geographic differentiation (interpopulational differences in growth rates could reflect either genetic differences or phenotypic responses to environmental variables), and (2) the sensitivity of growth rate to environmental variation can itself be regarded as a life history trait subject to natural selection (Sinervo and Adolph, 1989).

Temperature undoubtedly has a complex relationship with growth; reptiles respond to their external environment through behavioural adjustments of their body temperature (Andrews, 1982). Behavioural thermoregulation therefore tends to minimise seasonal and geographic differences in the body temperatures of lizards (Cowles and Bogert, 1944, Hertz and Huey, 1981). However, the amount of time per day (and per year) during which lizards can maintain high body temperatures can vary substantially, both geographically and seasonally (e.g. Van Damme, Bauwens and Verheyen, 1987; Grant and Dunham, 1988; Tsuji, 1988a; 1988b; Adolph and Porter, 1993; 1996).

Maintaining high body temperatures maximises many physiological processes including digestive rate and efficiency (e.g. Andrews, 1982; Waldschmidt, Jones and Porter, 1986; Van Damme, Bauwens and Verheyen, 1991) and food intake (Avery, Bedford and Newcombe, 1982;

Van Damme, Bauwens, and Verheyen, 1991). These processes are likely to have important impacts on growth rate. Thus, lizards that are prevented from attaining their selected body temperatures are likely to display reduced growth rates (Andrews, 1982). Consequently, thermal environments may be an important proximate source of variation in the growth rates of lizards (Sinervo and Adolph, 1994).

In association with its wide distribution in Tasmania, *N. ocellatus* displays considerable variation in life history characteristics, such as age and size at sexual maturity, longevity, adult body size, fecundity and growth rate across its range (Chapters 4 and 5). The purpose of the following experiments was to identify the relative importance of the source population and growth environment as contributors towards variation in growth rate. Growth rate was chosen as the focus of study for three reasons:

1. variation in this trait ultimately has direct consequences for other life history traits, especially adult body size, age and size at maturity (all of these have been observed to vary in *N. ocellatus*);
2. natural selection will tend to act primarily on growth rate early in life for species in which size at first breeding is important and may determine lifetime fecundity (Halliday and Verrell, 1988); and
3. growth rate is an index of physiological performance (including many whole body performances) that is correlated with fitness (Huey and Stevenson, 1979; Andrews, 1982; Christian, 1986).

In this chapter two experiments are described. In the first, conducted during 1994/1995, the importance of thermal opportunity to the growth rate of juvenile *N. ocellatus* was examined in the laboratory. In nature, *N. ocellatus* maintains a relatively high, constant body temperature when active through shuttling heliothermic activity, and low body temperatures while inactive (unpublished data). Although previous studies investigating lizard growth employed constant temperatures during normal activity, an ecologically realistic laboratory study should employ a cycling thermal regime in which lizards are able to behaviourally thermoregulate (Beuchat, 1988; Sinervo and Adolph,

1989). In the present study, lizard activity (and therefore the amount of time they were able to maintain high body temperatures) was varied between experimental groups by controlling access to basking opportunities. The following major questions were addressed:

1. is growth rate of newborn lizards related to access to basking opportunities?; and
2. do newborns from cold climates (Central Plateau study site), through previous strong selective pressures on growth rate, have a greater growth rate than their counterparts from milder climates (Orford study site) at any given temperature?

In the second experiment, conducted during 1996/1997, newborn lizards from the two study sites were transplanted to two sets of outdoor enclosures located in environments that provided conditions similar to those experienced at the study sites. Three major questions were addressed:

1. how does the thermal environment affect early growth rate?;
2. does growth vary during the season?; and
3. do newborns from the two populations differ in their growth responses?

6.2 Materials and methods

6.2.1 Experiment 1: basking opportunity in the laboratory

Gravid *N. ocellatus* close to parturition were collected from the two study sites. Variation in parturition dates of females at the two sites (Chapter 3), necessitated collections over two time periods. Collection took place in December 1994 at the Orford study site and late December 1994 to January 1995 at the Central Plateau study site.

Gravid animals were moved to the laboratory and placed under standard conditions (14L:10 D and 12 hour access to heat source) with feeding and

other conditions as described in Chapter 2. Cages were checked daily for newborns. Females that had given birth were removed from their cage and weighed (± 0.1 mg) and SVL measured (± 1 mm). Females were also palpated to confirm that parturition was completed. Full details of females caught, including size, clutch details, number of young per clutch, and relative clutch mass are included in Chapter 4 as part of an expanded data set.

Newborns were removed and SVL and total length measured (± 0.05 mm), weighed (± 0.1 mg), and each lizard was given a unique toe clip for permanent identification. They were then immediately placed into one of three experimental regimes: 5, 8, and 12 hour access to basking lights (25 W enclosed globe above a basking surface) which provided a thermal gradient within each cage (30+ °C immediately below the basking light and 15 °C in the rest of the cage) . Lizards were randomly allocated to these treatments within the constraint that clutches were split evenly between treatments. They were measured (± 0.05 mm) on 6 occasions during approximately the first 90 days of growth.

Lizards were housed in plastic terraria (30 x 20 x 10 cm) with a maximum of 15 individuals in any terrarium; all appeared to gain equal access to basking surfaces. Lizards were fed daily on a combination of live food commercially available for use in feeding tropical fish. This included blackworms (annelids) and live whiteworms (nematodes) mixed with calcium lactate (as suggested by Langerwerf, 1980) and a multivitamin mix. Equal amounts of food were given to each cage and food was in excess for a period of approximately one hour at which time lizards had ceased to be interested in feeding. Standard lighting of 14L:10D was maintained for all treatments throughout the experiment. Additional overhead UV lighting was provided, and vitamin D3 was added to their water (as suggested by Langerwerf, 1980).

6.2.2 Experiment 2: Growth rates in outdoor enclosures at two altitudes

Gravid *N. ocellatus* were caught from the Orford and Central Plateau study sites. Lizards were caught in the final few weeks of pregnancy at each site: late December 1996/early January 1997 for the Orford population and late January/early February 1997 at the Central Plateau study site. Females were housed in pairs in plastic terraria (30 cm x 20 cm x 10 cm) under standard light conditions (14L: 10D) with 12 hours of basking opportunity until parturition. No agonistic encounters were observed between female lizards, and they generally took advantage of the basking opportunities. Lizards were fed three times weekly with mealworms and soft fruit and water were available *ad libitum*.

Cages were checked daily for newborns. Females that had given birth were removed from their cage and weighed (± 0.1 mg), SVL measured (± 1 mm), and palpated to confirm that parturition was completed. Full details of females caught, including size, clutch details, number of young per clutch, and relative clutch mass are included in Chapter 4 as part of an expanded data set.

At birth the following details were taken from each newborn: SVL (± 0.05 mm), total length (± 0.1 mm) and weight (± 0.01 mg). Each lizard was then given a unique toe-clip for permanent identification. Each newborn lizard was sexed within the first few days. Sexing of hatchling scincid lizards has rarely been carried out. Several methods were initially tried, including that of Harlow (1996) and finally the method of Olsson (pers. demonstration). This method involved everting the hemipenes (when present) with a pair of modified forceps. The tips were bent at an angle of approximately 45 °C providing a V-shape which could then be rolled along the tail to evert the hemipenes. This method worked well and obvious hemipenes were everted on males. Some bruising resulted. Subsequently, simple eversion of hemipenes by fingers under a desk dissecting lens was tried and found to be as effective. Lizards up to 2 months old were resexed and no errors were found.

Within 12 hours of birth the performance of the juveniles was assessed using sprint speed as a measure of whole body performance. Toe-clipping does not affect sprint speed (Huey, Overall and Newman, 1990; Dodd, 1993). The lizards were placed in a sealed container within a water bath held at 27 ± 0.1 °C for 20 minutes. This is the temperature at which sprint speed in maximum (J. Melville, unpublished data). They were then run down a heated (27 ± 0.1 °C) "racetrack" two metres in length. The time taken was recorded by three infrared lightbeams held 50 cm apart and linked to a Macintosh computer. Each run generated two times (one for each of the 50 cm sections). Lizards were sprinted down the track twice in rapid succession and the fastest of the four times obtained was chosen as the best representative of maximum sprint speed. Each run was given a rating, where 1 represented a good run, 2 an acceptable run, and 3 a poor run. A run was rated as "good" when the lizard needed little encouragement to run down the track and there was no stopping for the length of the track; an "acceptable" run had some slowing and the lizard needed further encouragement to run down the track and "poor" runs resulted when the lizard either refused to run, stopped, or ran the wrong way. For the purposes of analyses any run that was given a rating of 3 was excluded. The ratings were independent of the actual sprint time.

Following measurement and performance testing, lizards were randomly assigned to one of two sets of outdoor enclosures between the sites (see below). All lizards were released in the outdoor enclosures within two days of birth. On three occasions during the experiment, all lizards were recaptured and remeasured (± 0.05 mm) and weighed (± 0.1 mg). The enclosures were systematically searched and all surviving lizards removed and transported to the laboratory for measurements. On each of these occasions the enclosures were searched thoroughly over a two day period to ensure that all lizards were recaptured. Following measurement, lizards were immediately returned to their enclosure.

6.2.2.1 Location and construction of outdoor enclosures

Five outdoor enclosures were constructed at each of two sites. The first site (hereafter referred to as the mountain site) was on Mt Wellington at an altitude of 1140 metres above sea level (Figure 6.1). This site represents the upper limit of the altitudinal range of natural populations of *N. ocellatus* on Mt Wellington (pers. obs.). At this site, *N. ocellatus* displays life history characteristics similar to those on the Central Plateau.

Figure 6.1. Outdoor enclosures at the mountain site. Each enclosure measures 2 metres in diameter.

At this site, adult body size is large and the reproductive cycle is delayed compared to lowland populations (pers. obs., unpublished data). At this altitude *N. ocellatus* occurs sympatrically with the generalist species *N. metallicus* and the alpine specialist species, the southern snow skink

Niveoscincus microlepidotus. At higher altitudes only *N. microlepidotus* is present. The second site (hereafter referred to as the lowland site) was a lowland area within a disused gravel quarry near the University of Tasmania and was at an altitude of 200 metres above sea level. *Niveoscincus ocellatus* occurs naturally at this site and displays life history characteristics typical of the Orford study site: small adult body size and non-delayed reproductive cycles (pers. obs.; unpublished data).

The enclosures were constructed of 50 cm tall fibreglass sheeting and each measured approximately 2 metres in diameter. Each enclosure was supplied with similar amounts of rock cover and water was provided *ad libitum*. At each site five enclosures were used and up to 18 lizards were placed in each enclosure. Lizards of the same age (and therefore approximately the same size) were placed in each cage to minimise competition between different size classes. This was achieved by filling one cage at a time to capacity. Some cages contained individuals from different populations. After recaptures and measurement, some lizards were re-assigned to maintain the same numbers of lizards in each enclosure. This density is greater than natural densities; however, supplemental food was supplied three times per week. Tinned cat food and various soft fruits were added liberally to each cage. These food types served both as a direct food source and as an attractant for flying insects. Observations of the lizards revealed that there were adequate basking and shelter sites for all lizards and few agonistic interactions were observed. A temperature data logger (Onset data logger, ± 0.1 °C) was placed under rock cover inside one of the enclosures at each site and were programmed to record the temperature every thirty minutes.

6.2.3 Growth rate analyses

In both experiments growth rates were analysed in a similar manner. Snout vent length (SVL), total length and initial weight were taken at birth and repeated at regular intervals. Growth rates of reptiles are most commonly expressed as rates of change in mass or SVL (Andrews, 1982; Sinervo and Adolph, 1989). In this study, snout vent length is used

because changes in mass may reflect recent nutritional history and changes in intestinal contents rather than a change in body size (Dunham, 1978). Snout vent length has a lower error variance associated with its measurement and provides a more direct measure of actual growth. Niewiarowski (1995) and Sorci, Clobert and Belichon (1996) both used Δ SVL/Δ time, and importantly growth rates have been shown to be linear early in life under constant conditions and over short time periods.

6.2.3.1 Analysis of Experiment 1

In Experiment 1, growth rates were linear throughout the growth period (Figure 6.2). Growth was calculated as the linear regression of size and time. The slopes of the regressions provided an estimate of the growth rate (mm/day) of individual lizards and were used as variates in analysis of variance (ANOVA) (Sinervo and Adolph, 1989; Sinervo and Adolph, 1994). The linear description of growth during the growth period was extremely good ($r = 0.846 - 0.995$). Initially, the relationship between growth rate and initial size was examined. No relationship was found ($P > 0.1$ in all cases) so analysis of covariance (with the initial snout vent length as the covariate) was unjustified (Smith, Ballinger and Nietfeldt, 1994). Individuals that did not survive, lost substantial weight, or were unhealthy in the first 60 days of the growth period, were excluded from the analysis. Comparisons between populations and treatments were made using a two way ANOVA. Although the populations gave birth at different times, the conditions in the laboratory were the same throughout the experiment, so direct comparisons were possible. Investigation of the maternal influence on growth rate was possible for the Orford population, but not for the Central Plateau because of low survival for the Central Plateau newborns and because the identity of the mother was not always known in this group as several mothers gave birth in the same cage, the same day. In the case of the Orford population, only seven females gave birth to three or more young (therefore providing 1 newborn per treatment) that survived for the duration of the

experiment. The maternal influence on growth rate was analysed by two way ANOVA with the treatment and the mother as the factors.

6.2.3.2 Analysis of Experiment 2

In Experiment 2, growth rates for each individual were estimated for each time period as the change in snout vent length (Δ mm) divided by the number of days since last measure (Δ days) to yield a growth rate of mm/day. Similarly to Sinervo and Adolph (1989) and Sinervo and Adolph (1994) these growth rates were used as variates in analyses of variance. Juveniles sourced from each population were remeasured three times, thus providing three growth periods. Growth period 1 for the Central Plateau population was very short for some individuals because they were recaptured soon after they were released for the first time, so results for this period should be treated with some caution.

Intrapopulational differences for each population were examined for each growth period by one way ANOVA. Although interpopulational comparisons of growth rate were possible in Experiment 1 because the conditions were constant, growth comparisons in Experiment 2 were more problematical because the animals were placed in the enclosures at different times and thus experienced different conditions for growth. However, because growth is linear early in life, it may be compared between similar time periods (even when lizard age differs slightly) when growth is expressed as the change in size (Δ SVL)/change in time (Δ time). Interpopulational comparisons were thus possible for growth periods 2 and 3 only, by two way ANOVA with source population and enclosure site as the factors. The influence of sex on growth was analysed with a two way ANOVA with sex and enclosure site as the factors within each source population.

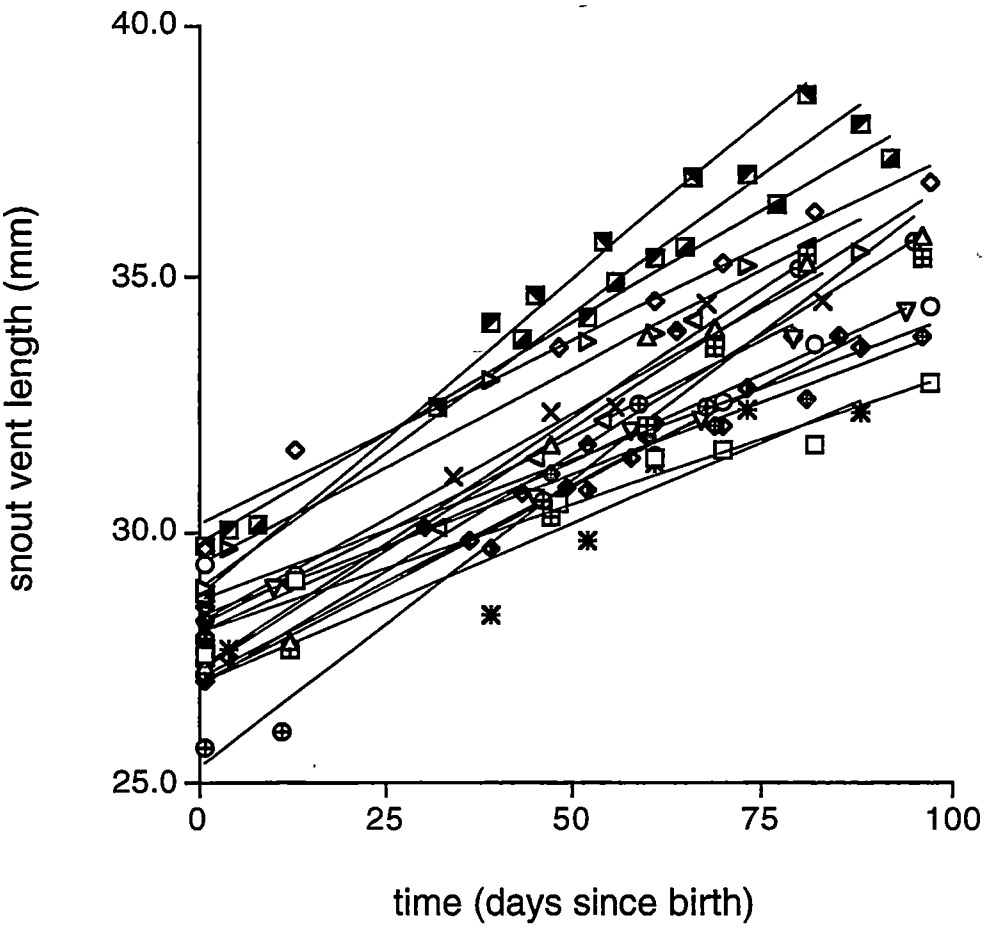

Figure 6.2. Growth of juvenile *Niveoscincus ocellatus* in the laboratory. This example is taken from the Orford population provided with 8 hours access to potential basking. Growth was calculated as the slope of size against time. Each line represents a linear regression of snout vent length and time for an individual lizard. The linear regression provided an excellent fit (r = 0.956-0.998).

6.3 Results

6.3.1 Experiment 1: basking opportunity in the laboratory

6.3.1.1 Newborn characteristics

Table 6.1 summarises the characteristics at birth of all juveniles that were used in the experiment. All newborn traits differed significantly between sites: snout vent length ($F_{1,137} = 71.046$; $P < 0.001$); total length ($F_{1,136} = 11.340$; $P < 0.005$); weight ($F_{1,136} = 61.358$; $P < 0.001$), and condition ($F_{1,136} = 40.306$; $P < 0.001$).

Table 6.1. Newborn *Niveoscincus ocellatus* characteristics for Orford and the Central Plateau populations from 1994/1995. Values are means ± standard error. Sample sizes are indicated in parentheses. (* denotes significance, $P < 0.05$)

juvenile characteristic	Orford	Central Plateau	sig
birth dates	1 Jan - 2 Feb 1995	20 Jan - 14 Feb 1995	
snout vent length (mm)	28.3 ± 0.11 (79)	29.6 ± 0.10 (60)	*
total length (mm)	64.9 ± 0.33 (79)	66.5 ± 0.33 (59)	*
weight (mg)	459 ± 6.0 (79)	535 ± 8.0 (59)	*
condition (mg/mm)	16.3 ± 0.17 (79)	18.1 ± 0.23 (59)	*

6.3.1.2 Newborn growth rates

Figure 6.2 presents the growth pattern for juveniles born to Orford mothers provided with access to basking opportunity for 8 hours per day. The linear regression of juvenile size and time provided an excellent fit for the data (r = 0.956 - 0.998). Similarly, in all other treatments, growth rates of individual lizards were linear in all cases and the linear regression equation provided an excellent fit to the data (r > 0.9 in most cases). The growth rate for all groups is presented in Table 6.2. and illustrated in Figure 6.3. For juveniles from both sites, growth rate differed significantly with access to basking opportunity (Orford: $F_{2,50}$

= 31.451; $P < 0.001$; Central Plateau: $F_{2,20} = 16.207$; $P < 0.001$). Two way ANOVA revealed no difference between the two sources of juveniles ($F_{1,70} = 1.914$; $P > 0.1$) and no interaction between sources of juveniles and hours of basking ($F_{2,70} = 1.516$ $P > 0.1$). Analysis of maternal effects on growth revealed no effect of mother ($F_{6,22} = 4.548$; $P > 0.1$) and no interaction between mother and access to basking ($F_{12,22} = 6.065$; $P > 0.1$).

Table 6.2. Growth rates (mm/day) of juvenile *Niveoscincus ocellatus* for each treatment group. The growth rate was calculated from only those juveniles that survived in a healthy condition for ~ 60 days (see methods). Values are means ± standard errors. The number of surviving lizards used to calculate growth rate are indicated in parentheses.

access	Orford		Central Plateau	
to basking	growth (mm/day)	survival (%)	growth (mm/day)	survival (%)
5 hours	0.04 ± 0.003 (18)	67	0.03 ± 0.005 (10)	45
8 hours	0.07 ± 0.004 (17)	63	0.06 ± 0.005 (9)	47
12 hours	0.08 ± 0.005 (18)	72	0.09 ± 0.010 (6)	32

6.3.2 Experiment 2: Growth rates in outdoor enclosures at two altitudes

6.3.2.1 Newborn characteristics

The Orford females gave birth between January 1 and 5 February 1997, and the Central Plateau females gave birth between 5 February and 18 February 1997. Newborns from the Central Plateau population were larger and heavier at birth (snout vent length: $F_{1,265} = 74.445$; $P < 0.001$; weight: $F_{1,265} = 91.543$; $P < 0.001$) than Orford newborns, although they did not differ in total length ($F_{1,264} = 0.213$; $P > 0.1$). Newborns from the Central Plateau were also in better condition ($F_{1,265} = 68.945$; $P < 0.001$)

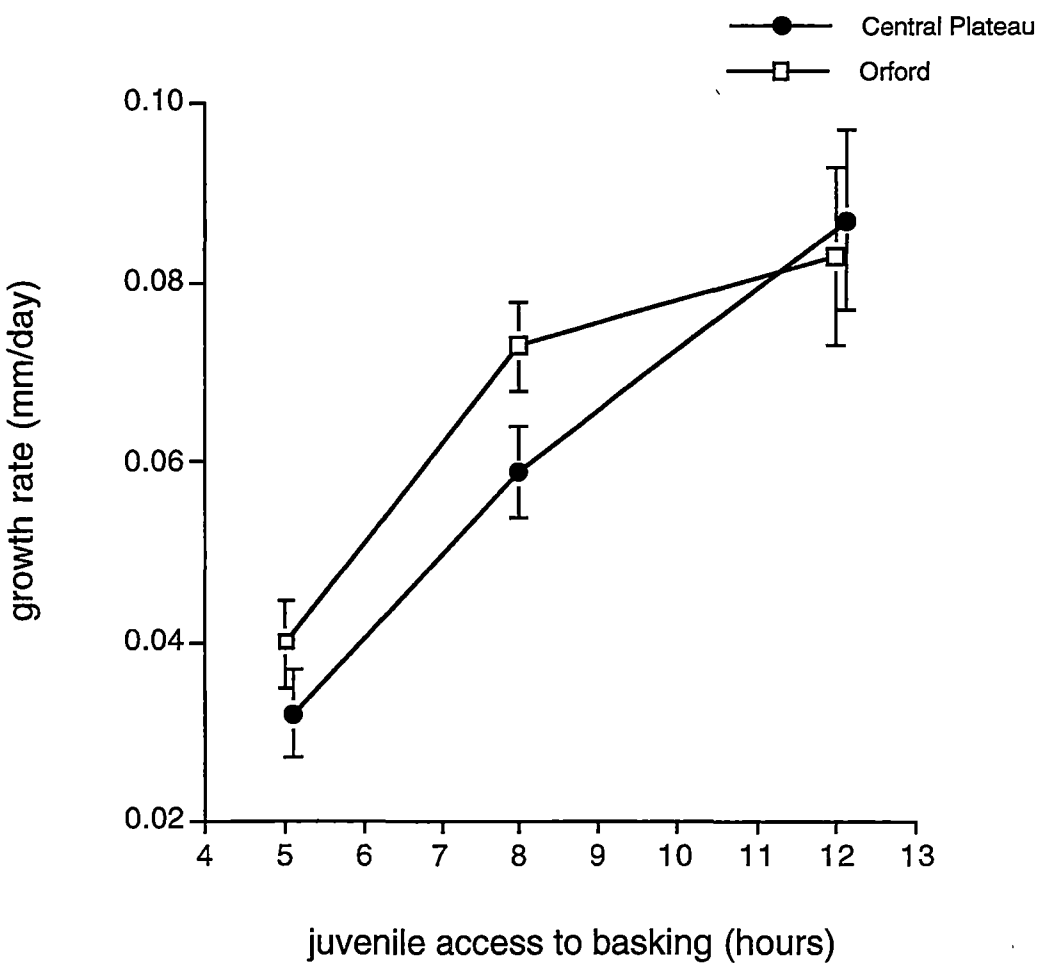

Figure 6.3. Mean growth rates (mm/day ± standard error) of juvenile *Niveoscincus ocellatus* in the laboratory. All values are calculated from least squares linear regression of snout vent length of individual juveniles over six time periods in 90-100 days (see Figure 6.2. and text). (Values for growth of Central Plateau juveniles at 5 and 12 hours offset for clarity only).

than those from Orford. Central Plateau newborns had a greater sprint speed at birth. Data for sprint speed were initially log-transformed for analysis to meet the assumptions of ANOVA ($F_{1,222} = 7.546$; $P < 0.01$).

Table 6.3. Newborn *Niveoscincus ocellatus* characteristics for Orford and the Central Plateau populations from 1996/1997. Values are means ± standard error. Sample sizes are indicated in parentheses. Birth date is number of days since January 1. (* denotes significance, $P < 0.05$)

character	Orford	Central Plateau	sig
average birth date	19.3 ± 0.74 (174)	42.6 ± 0.344 (93)	*
snout vent length (mm)	29.4 ± 0.06(174)	30.3 ± 0.07 (93)	*
total length (mm)	68.3 ± 0.20 (172)	68.2 ± 0.23 (93)	ns
initial mass (mg)	512.6 ± 3.88 (172)	574.6 ± 5.11 (93)	*
condition (mg/mm)	17.4 ± 0.11 (174)	18.9 ± 0.15 (93)	*
sex ratio	95 male: 77 female	51 male: 42 female	ns
sprint speed (m/sec)	0.94 ± 0.028 (145)	1.059 ± 0.042 (79)	*

There were more males born in both populations although the ratio did not differ significantly from a 1:1 ratio ($P > 0.1$ in both cases). There were no significant male and female differences in newborn characteristics for the Central Plateau ($P > 0.1$ for weight, snout vent length, total length and condition). Similarly males and females did not differ in snout vent length or total length ($P > 0.1$), but males were marginally heavier ($F_{1,170} = 3.431$; $P = 0.066$) and were in better condition ($F_{1,170} = 4.028$; $P = 0.046$). More males were born later than females, and there was a significant difference in the average birthday (days since January 1) of males and females from the Orford population ($F_{1,170} = 8.025$; $P < 0.01$), but the birth dates of males and females did not differ for the Central Plateau population.

6.3.2.2 Juvenile growth rate

6.3.2.2.1 Conditions at the outside enclosures

Thermal characteristics differed between the two enclosure sites. The lowland site was generally warmer, particularly later in the season (Figure 6.4 a and b). Both sites received regular rainfall; however, supplemental water was always available. Snow fell on several occasions at the mountain site during the final growth period, and conditions were well below freezing overnight. The time at which lizards were recaptured and measured is indicated on the Figures.

6.3.2.2.2 Intrapopulational variation between enclosure sites

Table 6.4 summarises the growth rate for both populations at the two sets of enclosures and Figures 6.5 and 6.6 illustrate snout vent length data. Figures 6.4 a and b indicates the period of measurement; growth period 1 occurred between birth and measurement 1, growth period 2 between measurement 1 and 2, and growth period 3 was between measurement 2 and 3. For simplicity, the initial data point for both populations was taken as the mean size at birth at the average birth date. Growth for each population showed similar trends, with rapid growth early and slower growth later in the season. Growth rate was significantly slower at the mountain site for each growth phase for both populations ($P < 0.001$) except during the first period of growth when Orford lizards grew more rapidly at the mountain site ($F_{1,135} = 4.463$; $P < 0.05$) and the Central Plateau lizards grew at similar rates at both sites ($F_{1,75} = 2.822$; $P > 0.05$). All analyses were also conducted with size at the start of the growth period as the covariate (Sinervo and Adolph, 1989; 1994); this did not change the conclusions presented above.

Growth rates showed no consistent relationship with sprint speed at birth. For the young derived from the Orford population, there was a significant negative relationship between sprint speed and growth rate in

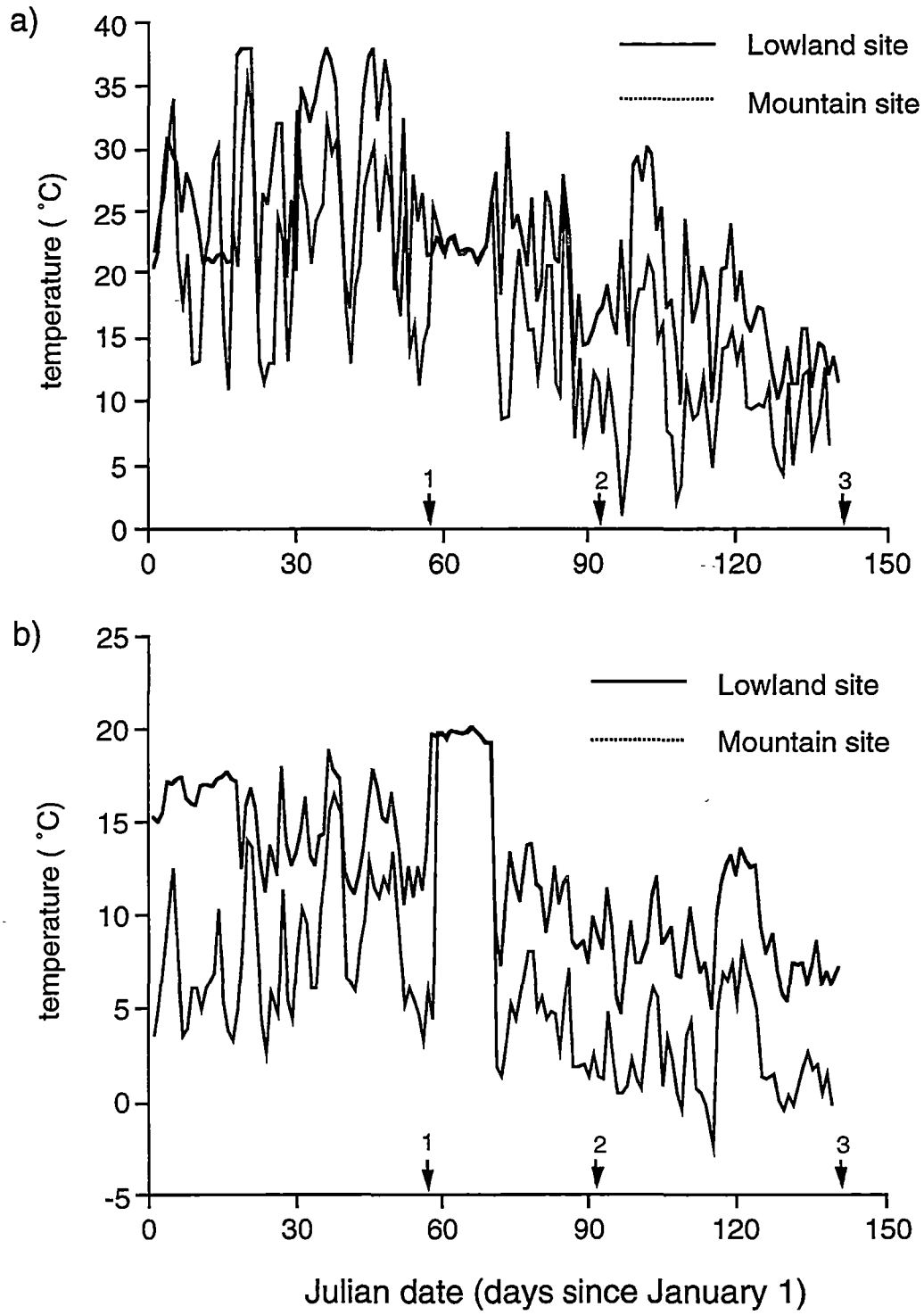

Figure 6.4. Thermal characteristics at the mountain and lowland enclosure sites based on data loggers placed inside one enclosure at each site; a. is maximum daily temperature recorded and b. is the daily minimum temperature.

Table 6.4. Growth and survival of juveniles at the two sets of outdoor enclosures sourced from Orford and the Central Plateau. Growth rates are expressed as means ± standard error. The number that survived (and % survival from the original number released) until the end of each of the growth periods is also indicated.

Source	Orford				Central Plateau			
Locality	mountain		lowland		mountain		lowland	
	growth rate (mm/day)	%survival	growth rate (mm/day)	%survival	growth rate (mm/day)	%survival	growth rate (mm/day)	%survival
Initial No.		85 (100%)		89 (100%)		46 (100%)		47 (100%)
period 1	0.06 ± 0.003	72 (85%)	0.05 ± 0.002	65 (73%)	0.06 ± 0.004	42 (91%)	0.07 ± 0.005	35 (74%)
period 2	0.01 ± 0.001	69 (81%)	0.03 ± 0.002	51 (57%)	0.01 ± 0.002	36 (78%)	0.03 ± 0.003	16 (34%)
period 3	0.00 ± 0.001	45 (53%)	0.02 ± 0.001	41 (46%)	0.00 ± 0.001	17 (37%)	0.01 ± 0.003	13 (28%)

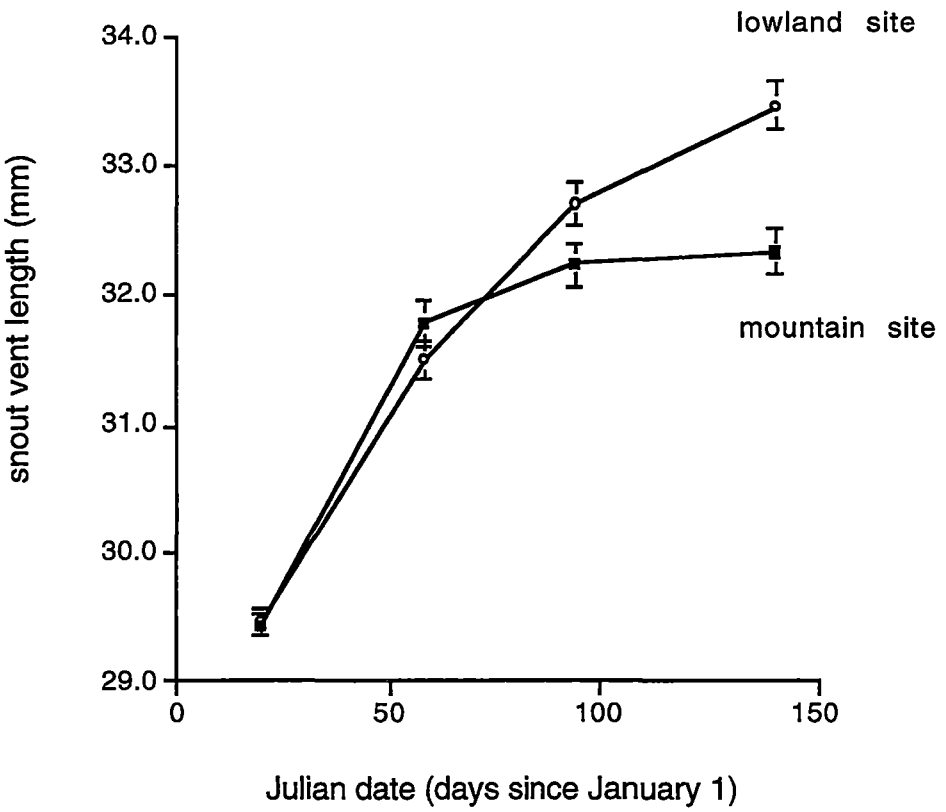

Figure 6.5. Comparison of growth rates of juvenile *Niveoscincus ocellatus* from Orford at the lowland and mountain enclosures. Values are mean snout vent length ± standard error.

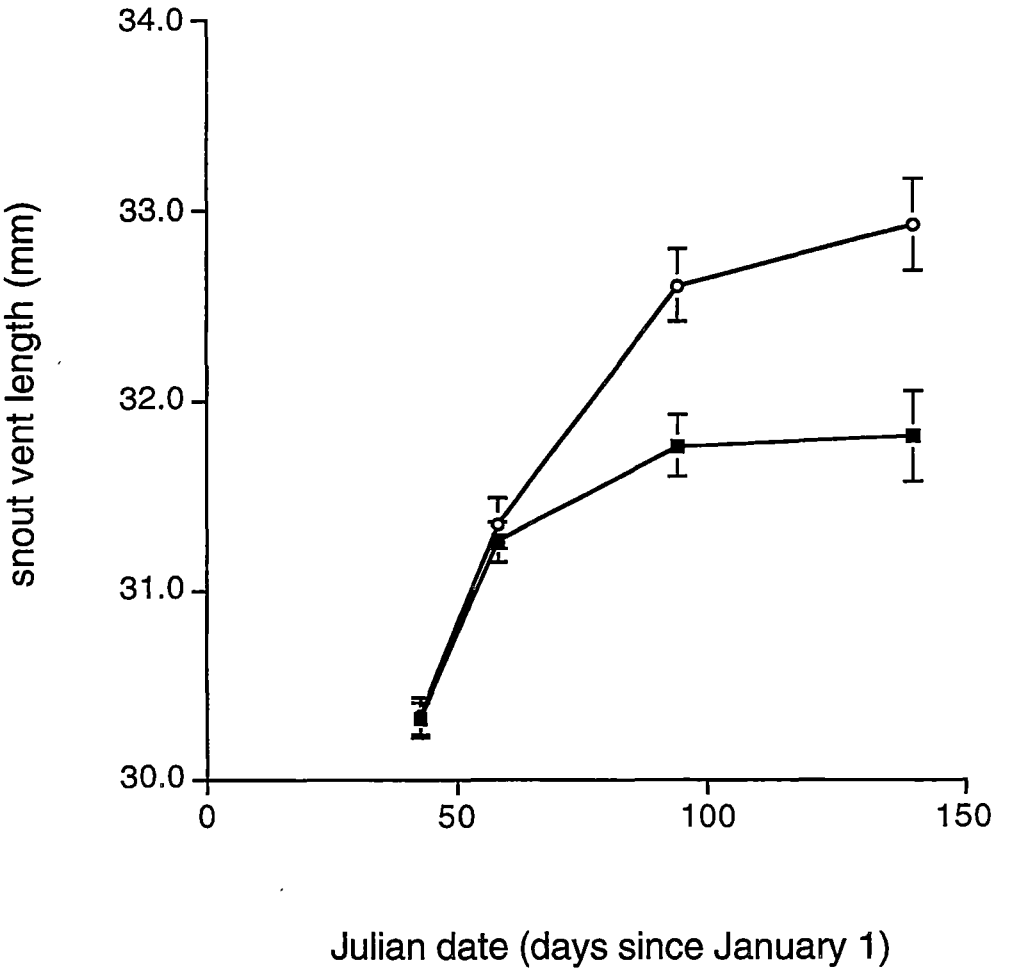

Figure 6.6. Comparison of growth rates of juvenile *Niveoscincus ocellatus* from the Central Plateau at the lowland and mountain enclosures. Values are mean snout vent length ± standard error.

growth period 1 at the lowland site (r = 0.339; $F_{1,54}$ = 8.155; $P < 0.001$), but no relationship at the mountain site. The young obtained from the Central Plateau population showed the opposite pattern, with a significant negative relationship at the highland site (r = 0.452; $F_{1,28}$ = 7.177; $P < 0.05$) and no relationship at the lowland site.

6.3.2.2.3 Interpopulational variation in growth rates

Growth rates of lizards are typically linear early in life (Andrews, 1982; Sorci, Clobert and Belichon, 1996) and under constant conditions are linear in *N. ocellatus* (Experiment 1) for up to 120 days. A comparison of growth rates between source populations is only justified for periods of growth in which the two populations experienced the same conditions for growth (see methods). Figure 6.7 shows the growth of juveniles sourced from both populations at the lowland site and Figure 6.8 the equivalent data for the mountain site of juveniles sourced from both populations during growth periods 2 and 3. Although the juveniles derived from the Orford mothers were larger during the two periods of growth at both sites than those derived from the Central Plateau, there is little difference in the growth rates. Two way ANOVA demonstrated that the two source populations showed similar growth patterns at each locality: growth period 2 (enclosure site; $F_{1,168}$ = 0.143; $P > 0.1$; enclosure site*locality; $F_{1,168}$ = 2.822; $P > 0.1$), growth period 3 (enclosure site; $F_{1,112}$ = 3.606; $P = 0.06$; enclosure site*locality; $F_{1,112}$ = 3.355; $P = 0.07$). In growth period 3, the results are only marginally non significant and further analysis revealed (ANOVA) that the only difference between the populations is at the lowland site where the Orford population grew slightly more rapidly ($F_{1,52}$ = 5.563; $P = 0.022$).

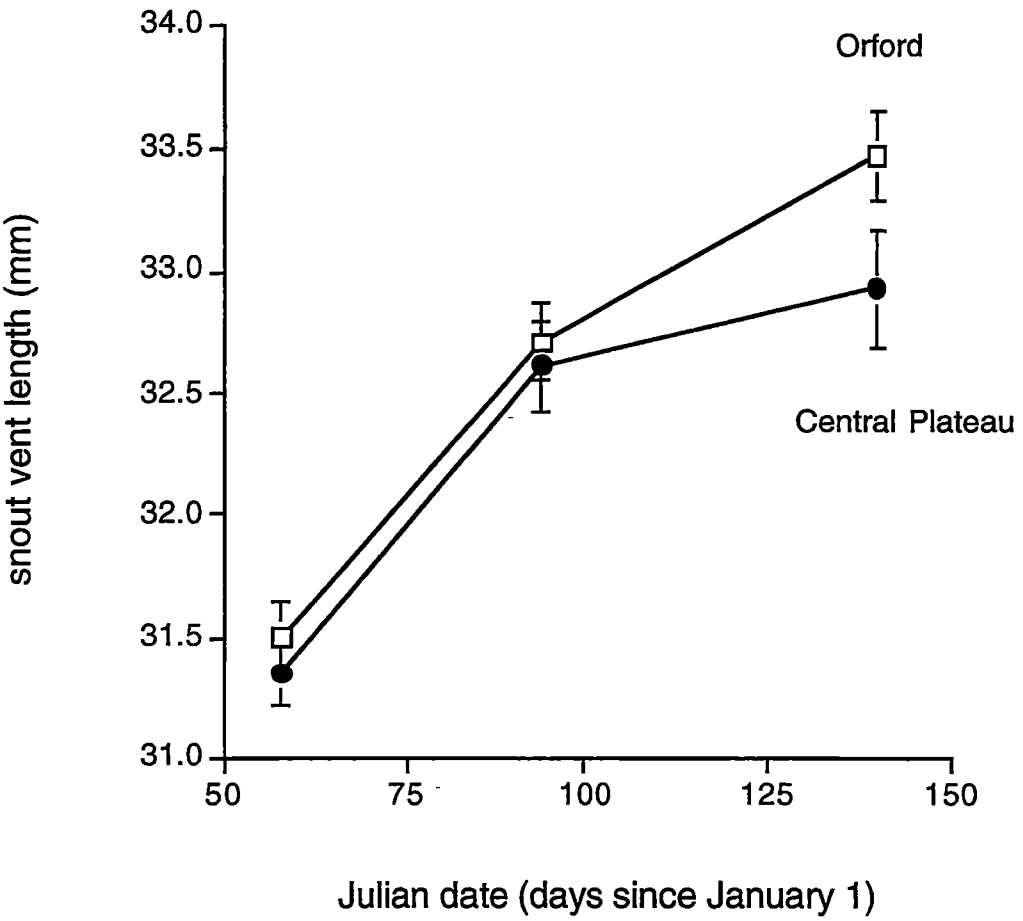

Figure 6.7. Comparison of growth rates of juvenile *Niveoscincus ocellatus* from the Central Plateau and Orford at the lowland site for the final two growth periods (see text for explanation). Values are mean snout vent length ± standard error.

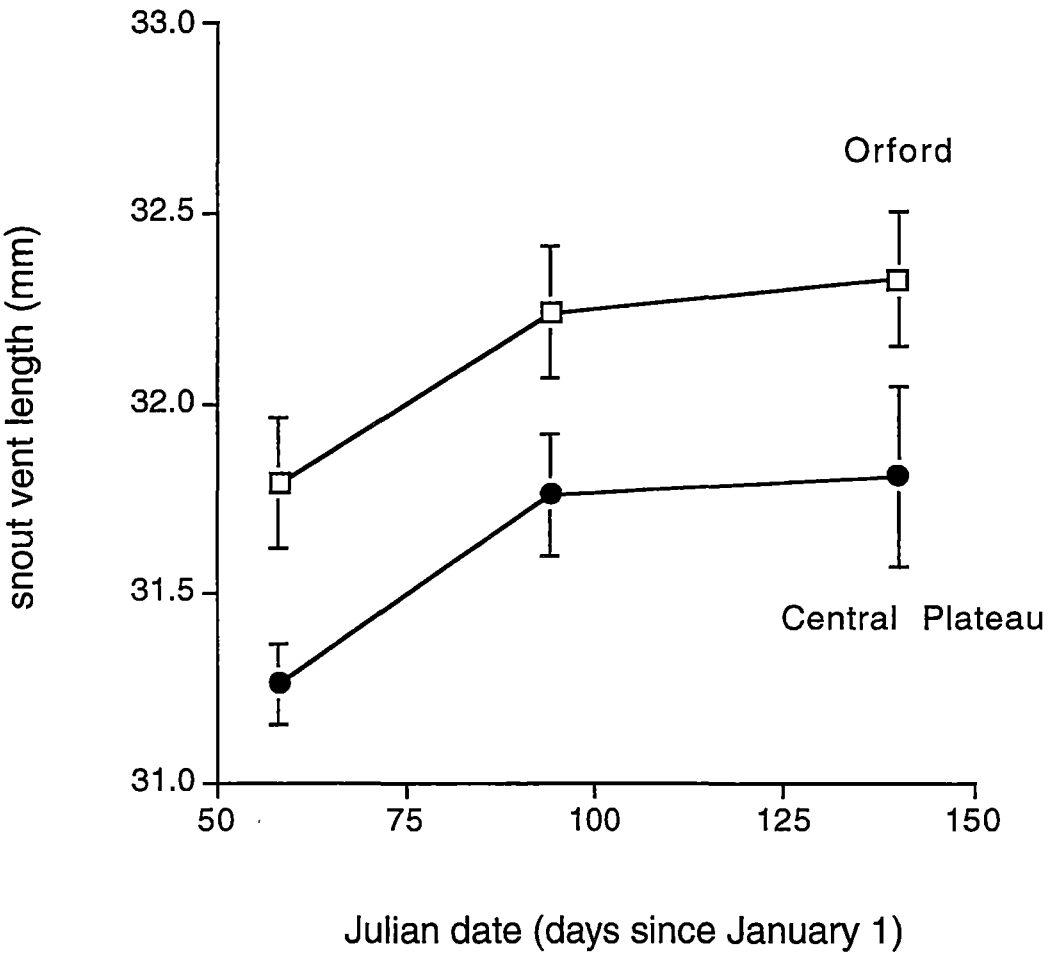

Figure 6.8. Comparison of growth rates of juvenile *Niveoscincus ocellatus* from the Central Plateau and Orford at the mountain site for the final two growth periods (see text for explanation). Values are mean snout vent length ± standard error.

6.3.2.2.4 Intersexual variation in growth rate

Two way ANOVA revealed that there were no intersexual differences in growth rate for the young derived from the Orford population ($P > 0.1$ in all cases) and there were no interactions between sex and locality for any growth period (growth period 1: $F_{1,131} = 3.322$; $P = 0.071$; growth period 2: $F_{1,131} = 3.215$; $P = 0.076$; growth period 3: $P > 0.1$)) (Figure 6.9 a and b). The juveniles derived from the Central Plateau population similarly showed no intersexual difference in growth rate ($P > 0.1$ in all cases) and little indication of interaction between sex and locality ($P > 0.1$ in growth periods 1 and 2; growth period 3: $F_{1,26} = 4.343$; $P = 0.047$) (Figure 6.10). Sample sizes were low for the Central Plateau during growth period 3 (lowland site: 5 males, 8 females: mountain site: 8 males, 9 females).

6.4 Discussion

6.4.1 Intrapopulational differences in growth rates

The thermal environment exerted a strong proximate influence on growth rate in newborn *N. ocellatus*. Similar effects have been reported previously (e.g. Sinervo and Adolph, 1989; 1994; Sinervo 1990a). In the laboratory study (Experiment 1), growth rate increased with increased access to basking opportunities. Observations of the juveniles revealed that, predictably, activity (basking/feeding/moving) was maintained while lizards had the ability to thermoregulate, and activity decreased when basking was not possible.

In the outdoor enclosures (Experiment 2) lizard growth responded to the thermal environment in a similar manner. Lizards from both populations displayed a higher growth rate at the warmer lowland enclosures and growth decreased at both enclosure sites later in the season, presumably as a result of both cooling weather conditions and decreasing day length. It may be assumed that the difference in growth rates between juveniles at the mountain and lowland enclosures is a consequence of the differences in the thermal environments. Other

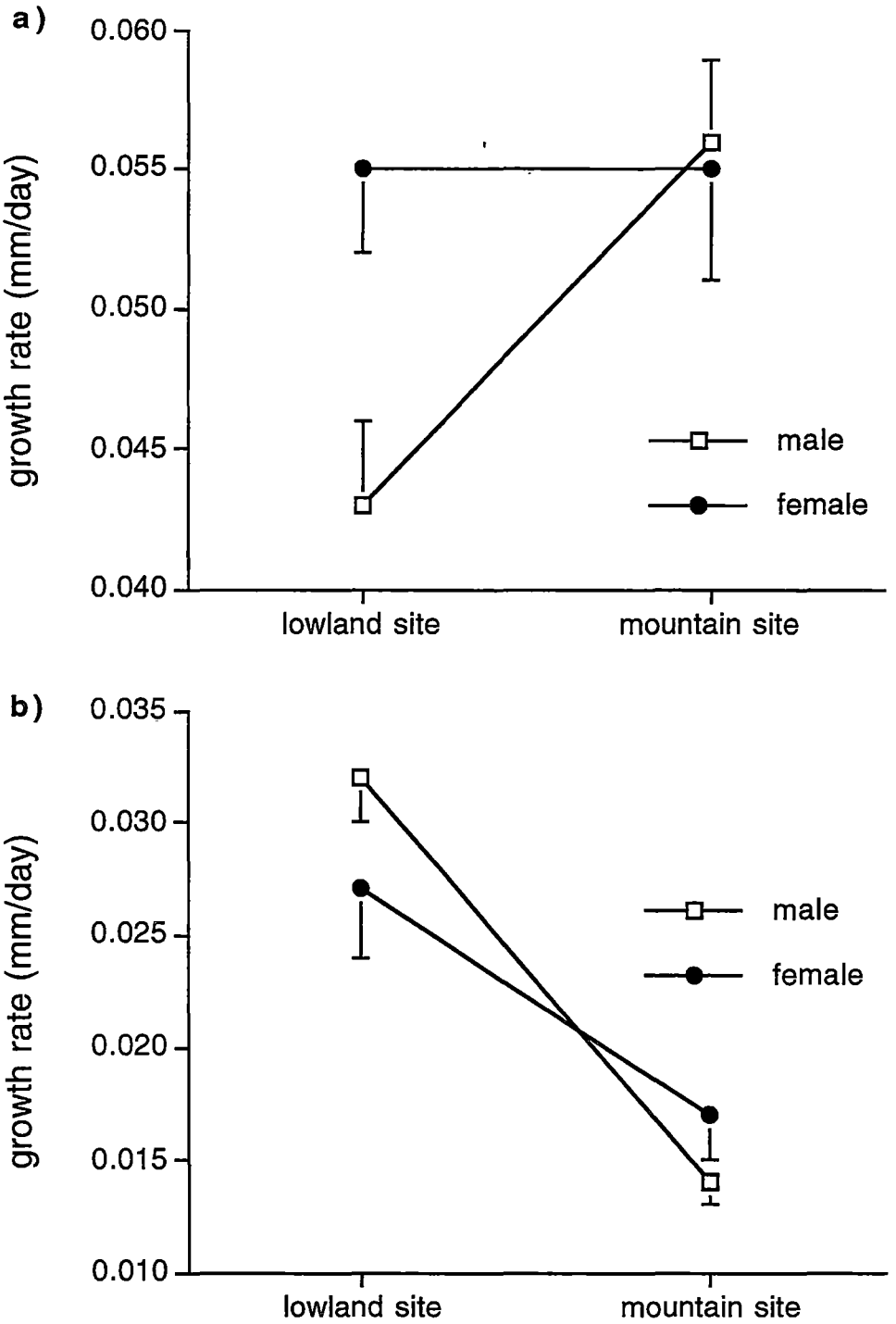

Figure 6.9. Intersexual differences in growth rates (mm/day ± standard error) of juvenile *Niveoscincus ocellatus* for the Orford population at the lowland and mountain sites (a. growth period 1; b. growth period 2; a, positive errors for males, negative errors for femaes; b, negative errors for males and females for clarity).

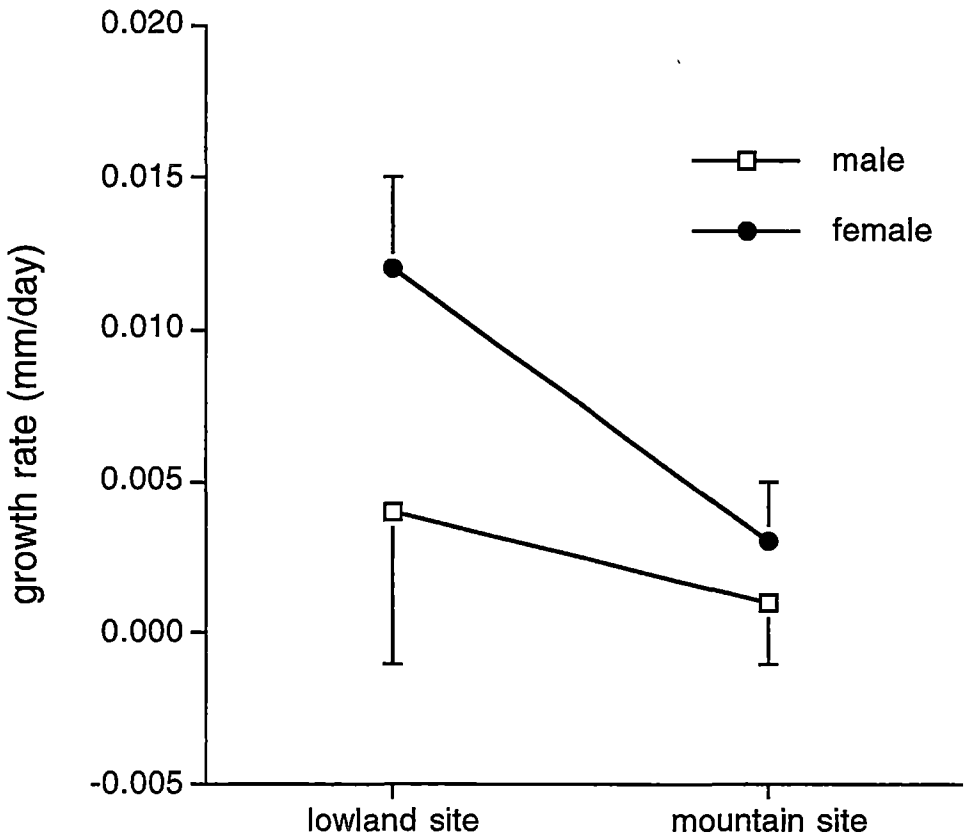

Figure 6.10. Intersexual differences in growth rates (mm/day ± standard error) of juvenile Niveoscincus ocellatus from the Central Plateau population for growth period 3. (negative errors for males, positive errors for females for clarity)

variables typically associated with variation in growth rates (food and water availability) were similar at both locations. All cages were supplied with food three times weekly and water was available *ad libitum*, and the number of basking and shelter sites were similar in all enclosures.

Sinervo and Adolph (1989; 1994) suggest that two mechanisms may underlie the dependence of growth rate on potential activity time. Firstly, lizards with more potential activity time are able to spend more time at their preferred body temperature. At preferred body temperatures, the rates of physiological processes such as food digestion, food assimilation, metabolism, and conversion to somatic tissue are typically higher than at lower body temperatures (Van Damme, Bauwens and Verheyen, 1991; Sinervo 1990a; Sinervo and Adolph, 1989; Waldschmidt, Jones and Porter, 1986).

Secondly, lizards with greater potential activity time have more time available for foraging and food intake (e.g. Avery, 1971; 1978; Huey and Bennett, 1987; Taylor, 1986a; Jones, Ballinger and Porter, 1987; Brown, 1991; Niewiarowski and Roosenburg, 1993; Olsson and Shine, 1997). In environments where lizards are less able to thermoregulate and maintain near optimum body temperatures, there may be a reduction in the time spent foraging, total distance moved, and encounter rates with potential prey (Avery, Bedford and Newcombe, 1982). In addition to more time for foraging (and presumably a greater food intake if food is equally available at all time periods), a high body temperature may also be important for prey handling efficiency and appetite (Avery, Bedford and Newcombe, 1982; Waldschmidt, Jones and Porter, 1986; Van Damme, Bauwens and Verheyen, 1991). In *N. ocellatus* feeding rate, appetite, prey handling ability and digestion rate are all enhanced at body temperatures close to optimum temperature, 28-31°C (Wapstra, 1993). Body temperatures during periods of inactivity (e.g. at night) have also been shown to affect growth rate through an influence on digestive processes (Christian, 1986).

It has been suggested that low growth rates in some populations of lizards result directly from either food limitation (food hypothesis),

thermal constraints that reduce activity time (thermal hypothesis) or both (Niewiarowski, 1995). In *N. ocellatus*, differences in growth rate are consistent with predictions that growth rate varies as a response to the opportunity for lizards to maintain preferred body temperatures. In both the field and laboratory experiments food was equally available to all groups of lizards and was unlikely to have directly limited growth rate. My data indicate that growth rates measured in the field cannot be used to infer genetic differences among populations because they may simply reflect geographic variation in thermal environments. My argument is further supported by the finding that no significant differences were detected in growth rates between the populations, either in the laboratory or in the outdoor enclosures when young from these populations were reared in the same conditions.

In addition to considerable variation between treatments within the laboratory, there was also large variation within treatments (Figure 6.2). Such intrapopulational variation in growth rates among individuals is commonly found (e.g. Andrews, 1982; Dunham 1978; Sinervo and Adolph, 1989; Sinervo 1990a). Although high variability in growth rates of reptiles has hindered the search for inter- and intraspecific patterns of variation it does represent an important adaptive trait (Andrews, 1982). This variation has been attributed to genetic factors, particularly family membership (Sinervo and Adolph, 1989; Sinervo 1990a; 1990b), and differences in temperature selection and activity by individuals (Sinervo, 1990a). Sinervo and Adolph (1989) found that variation in growth rates attributable to family effects (sibships) was double that induced by the thermal environment. No maternal effects were found in this study, although low clutch sizes for the Orford population and high mortality for the Central Plateau population make analyses unreliable. Maternal effects are likely to be reflected in initial hatchling size (Sinervo, 1990a), but the data can be adjusted for this statistically using ANCOVA with the initial size of offspring as the covariate (Niewiarowski and Roosenburg, 1993). Since there was no relationship between initial size and subsequent growth rate in *N. ocellatus*, ANCOVA was unjustified in the analyses of my data.

6.4.2 Interpopulational differences in growth rates

Identifying sources of life history variation between species and populations has been a challenge in the development of life history theory. The use of reciprocal transplant experiments and common garden experiments to reveal inter- and intraspecific sources of variation in life history traits (including growth rate) is relatively new to herpetology and most work has been restricted to North American iguanids in the genus *Sceloporus*.

In these studies, and in my study of *N. ocellatus*, it has been hypothesised that cold climate populations (usually high altitude or latitude) may have evolved a more rapid growth rate. "Latitudinal" or "altitudinal" compensation may result when growth rate evolves in a direction opposite to that of the observed phenotypic pattern to partially counteract the negative influence of the physical environment on growth; this phenomenon is termed "countergradient variation" (Levins, 1969; Conover and Present 1990). In these situations individuals from high latitudes or altitudes may be expected to grow faster than those from low latitudes/altitudes when compared at the same temperature (Schultz, Reynolds and Conover, 1996). Similarly, individuals from regions with short activity seasons may compensate by increasing growth rate at temperatures most commonly encountered in the field (Conover and Present, 1990) which in reptiles is likely to correspond to temperatures at or near their preferred body temperature. Again, high altitude/latitude species would be expected to have a higher growth rate in any given conditions.

Investigation of intraspecific variation in growth rates of ectothermic groups confirms that growth rates may be subject to selection. For example, Berven, Gill and Smith-Gill (1979) and Berven and Gill (1983) found differences in reaction norms for tadpole growth and differentiation among populations from different altitudes. Tadpoles tended to grow faster in water that was of similar temperature to their natural habitats. Reciprocal transplant experiments and common garden

laboratory investigations revealed strong proximate influences on growth, with an underlying genetic component (Berven, 1982). Similar work on the fish species *Menidia menidia* and *Fundulus heteroclitis* found genetic differences in growth rate between individuals from different latitudes (Conover and Present, 1990; Schultz, Reynolds and Conover, 1996 respectively).

However, evidence for a consistent underlying genetic difference in growth rates between populations of geographically widespread lizard species is less convincing (see below). In the present study, juvenile *N. ocellatus* from the Central Plateau population did not show a higher growth rate than juveniles from the Orford population in any of the treatments, either in the laboratory or in the field enclosures. While all studies of growth rate to date (including this one) have revealed strong proximate effects on growth (Sinervo and Adolph, 1994), many have revealed differences in growth rates that are likely to be partly genetic and consistent with the predictions made above (e.g. Sinervo and Adolph, 1989; 1994; Sinervo 1990a; 1990b). For example, Ballinger (1979) found that juvenile *S. jarrovi* taken from high elevation sites and placed in enclosures at low altitude grew more quickly in the enclosures than transplants from low elevation sites. Ferguson and Brockman (1980) found both interspecific differences between *S. undulatus* and *S. graciosus* and intraspecific differences in growth rate between populations of *S. undulatus*. Similarly, Ferguson and Talent (1993) found differences in growth rates of populations of *S. undulatus* raised in common laboratory conditions. Niewiarowski and Roosenburg (1993) used reciprocal transplant experiments to reveal population level differences in growth rates in *S. undulatus*. Niewiarowski (1995) argues that evidence is accumulating (at least in *S. undulatus*) to justify assumptions of genetic divergence (for specific populations) required by adaptive hypotheses explaining geographic differences in growth rate and other life history traits.

There is less published work on other groups of lizards to confirm the generality of the findings reported for *Sceloporus*. Schwarzkopf (1996b) has recently examined geographic variation in the widespread Australian

skink *Eulamprus quoyii*. In this species, there is evidence that differences in growth rates of juveniles raised in the laboratory are influenced by a variety of factors, including the origin of the mothers. Similarly, Mateo and Castanet (1994) found significant population level differences in the growth rate of *Lacerta lepida* in the laboratory.

While all the studies above have revealed a genetic basis for differences in growth rates, this is not always so. Even within *Sceloporus* , for which there has frequently been demonstrated an underlying genetic component to variation in growth rates, the results differ. For example, Sinervo and Adolph (1994) found no intraspecific genetic difference in growth rate between populations of *S. occidentalis* or *S. graciosus* from different elevations and Smith, Ballinger and Nietfeldt (1994) similarly found no difference between populations of *S. jarrovi* from different altitudes. Sorci, Clobert and Belichon (1996) likewise were unable to detect intraspecific differences in growth rate of *Lacerta vivipara* from populations from different elevations raised under standard conditions in the laboratory. The results from these studies are more consistent with my own results. I was unable to detect evidence for a genetic basis for differences (at the population level) in growth rates between populations of *N. ocellatus*.

Selection may have minimised differences between populations if fast growth is favoured equally at both sites. For example fast growth may be favoured at the Central Plateau to counter the poor growth potential at this site (see above), while selection for rapid maturity may favour fast growth at Orford. Alternatively, growth rate may not evolve between populations because there are trade-offs between growth and other life history traits. There may be, for example, a negative correlation between growth capacity and survivorship under certain environmental conditions (Conover and Present, 1990; Forsman and Lindell, 1991; Adolph and Porter, 1993; Sorci, Clobert and Belichon, 1996).

For instance, juvenile *N. ocellatus* from the Central Plateau study site may not show an enhanced growth rate because they may have a greater dependency on stored energy for overwinter survival than their

counterparts from lowland/warmer sites. Energy that is stored for basic maintenance and overwinter survival by high altitude/cold climate individuals may be devoted to somatic growth by individuals from more benign climates. Sinervo and Adolph (1989) argued that energy allocated to survival rather than growth may be appropriate for juveniles faced with (1) only a few weeks of warm weather after they hatch, and (2) a lengthy overwintering period. Both these conditions are faced by juvenile *N. ocellatus* from the Central Plateau. Although trade-offs have received considerable attention in the life history literature (see Stearns 1989b; 1992; Roff, 1992 for recent reviews), the trade-off between juvenile growth and survival has received little attention. Further work on the use of available energy (growth versus maintenance and survival) between populations from different environments is required.

Alternatively, the absence of genetically based differences may result from gene flow between populations. There is evidence in skinks that differences in life history traits, including growth rate, between populations may be explained without invoking a genetic basis for them. For example, in *Eulamprus tympanum* Rohr (1997) found significant differences in life history traits between two populations separated by only a short distance (6 km) and where there is likely to have been considerable gene flow between them. However, it is unlikely that there is significant gene flow between the populations of *N. ocellatus* used in this study. The Central Plateau and Orford populations are separated by approximately 200 km and there is no continuous population of *N. ocellatus* over this distance. Furthermore, there is evidence that these populations have diverged genetically over time (Melville, unpublished data). Thus, the potential for evolved differences in life history traits (including growth rates) exists in this species, and particularly in the populations used in this study, but this does not appear to have translated into genetic differences in growth rates.

6.4.3 Consequences of growth differences

Growth rate is likely to affect fitness primarily through its effect on body size. Body size affects a variety of performance-related traits in lizards, including sprint speed (Sinervo and Adolph, 1989; Sinervo and Huey, 1990; Sinervo and Losos, 1991), ability to hold territories (e.g. Fox, 1978; Simon, 1975; Simon and Middendorf, 1980; Ferguson, Hughes and Brown, 1983; Stamps, 1983; 1988), dispersal distance (Doughty and Sinervo, 1994; Doughty, Sinervo and Burghardt, 1994), predator escape (Dunham, 1978) and survival during the active season and during winter (e.g. Ferguson and Fox, 1984; Olsson, 1992; Sinervo *et al.*, 1992; Sinervo and Adolph, 1994). An ability by larger juveniles to sprint more quickly may in turn affect their ability to capture prey and escape predators (Sinervo and Adolph, 1989). Thus, there is likely to be strong selective pressure on growth rate; either directly through selection on fast growing genotypes, or indirectly through maximised responsiveness to proximate environmental influences on growth rate.

Both growth rate and initial hatchling size affect body size at a given age (Sinervo and Adolph, 1994). Both these factors (but especially growth rate) are likely to influence the size of juvenile *N. ocellatus* at the Central Plateau and Orford study sites. Parturition occurs earlier at Orford than at the Central Plateau and this provides newborns from Orford with a longer potential growing season. Furthermore, this period corresponds to the period when growth is most rapid because of favourable thermal conditions (Figures 6.5 and 6.6). Conversely, the newborns from the Central Plateau study site have a shorter potential growing season because they are born later when thermal conditions are less favourable and moreover in a habitat where winter comes earlier. The differences in birth date and thermal conditions for growth are likely therefore to result in larger lizards for any given age at Orford than at the Central Plateau. These arguments assume that growth at the study sites shows a similar pattern to that obtained for the growth rates in the outdoor enclosures and that food is equally available at both sites. Differences in size are likely to increase even further because the period of winter inactivity is much longer at the Central Plateau than at the Orford study site (Figure

2.4; pers. obs.). The larger size of offspring borne to Central Plateau lizards may partially compensate for their reduced growth opportunities and may allow the species to occur in colder environments (see also discussion in Chapter 4).

Juvenile growth rate is also a major determinant of size and age at maturity (Andrews, 1982; Sinervo, 1990a; Adolph and Porter, 1996). Populations with a low growth rate will either mature at the same age but at a smaller size, or delay maturity until a later age and a larger size than populations with a more rapid growth rate (Adolph and Porter, 1996; Chapter 5). Maturation in temperate-zone lizards typically occurs in an integral number of years (Sinervo, 1990a). Age and size at maturity, and growth rate are presumably part of the co-evolved strategy of a genotype resulting from selective pressures imposed by environmental conditions (James, 1991b). In *N. ocellatus*, rapid growth in warm climates allows attainment of the minimum size for reproduction at the end of their second season (to produce their first clutch in their third season) while at the Central Plateau they are unable to reach this size within the same time period and thus mature at a later age and at a larger size (further discussion in Chapter 5). Demographic models predict that maturity will be delayed if growth is slow (Stearns 1977; 1992), especially if adult survival is high. At the Central Plateau, the combination of slow growth (this chapter) and high adult survival (Chapter 5) appears to select for delayed maturity at a larger size than at lowland sites. Growth rate differences between populations of *N. ocellatus* (as a result of thermal differences between sites) appear to lead to plastic responses in size and age at maturity, and thus two phenotypically plastic responses (growth rate and age and size at maturity) may be responsible for variation in life history traits observed between populations.

Chapter 7

The influence of maternal basking opportunities on juvenile phenotype, survival and growth rate in *Niveoscincus ocellatus*

7.1 Introduction

Many factors affect juvenile phenotype at birth. The environment in which a reptile embryo develops profoundly influences the morphology, physiology, behaviour, and survival of hatchling or newborn reptiles (e.g. Van Damme *et al.*, 1992; Miller, 1993; Shine and Harlow, 1993; Webb and Manolis, 1993; Shine 1995; Castilla and Swallow, 1996).

Most studies that have examined factors (especially incubation temperature) influencing embryo development in reptiles have concentrated on oviparous species. In viviparous species, conditions during gestation, particularly maternal thermoregulation, may also have important influences on embryonic development, development time, and juvenile phenotype. However, surprisingly few studies have examined the potential role of gestation environment in embryonic development of viviparous lizards (but see Beuchat, 1988; Shine and Harlow, 1993).

The temperature at which reptilian embryos are incubated can influence many aspects of development. Gestation usually proceeds more rapidly at higher temperatures (Beuchat, 1988; Van Damme *et al.*, 1992; Shine and Harlow, 1996). Temperature also affects juvenile phenotype in reptiles: turtles (De Souza and Vogt, 1994; Roosenburg and Kelley, 1996; Miller, 1993); crocodiles (Webb, Choquenot and Whitehead, 1986; Webb and Manolis, 1993), snakes (Burger, 1989; 1990), and lizards (Shine and Harlow, 1993; Shine, 1995; Shine and Harlow, 1996; Mathies and Andrews, 1997).

In viviparous species, the thermal environment experienced by embryos is influenced by the thermoregulatory behaviour of the female (Beuchat, 1988; Shine and Harlow, 1993). The thermoregulatory behaviour of females during pregnancy has been studied extensively (Schwarzkopf and Shine, 1991; Daut and Andrews, 1993; Lecomte, Clobert and Massot, 1993; Tosini and Avery, 1996 and references therein). While active, lizards will usually thermoregulate to maintain body temperatures at or near their preferred body temperature. However, the amount of time per day (or per year) during which lizards can maintain high body temperatures varies geographically and seasonally (Hertz and Huey, 1981; Hertz, Huey and Nevo, 1983; Grant and Dunham, 1988; 1990; Tsuji, 1988a; 1988b; Adolph and Porter, 1993; 1996). Thus, lizards occupying different environments are constrained in the amount of time they can be active. Activity time profoundly affects life history traits of adult reptiles (including lizards). For example, Adolph and Porter (1993) demonstrated that observed life history differences between populations of the lizard *Sceloporus undulatus* may be explained in part by the length of the activity season. However, the effects of different activity times by adult female lizards, or more specifically the influence of female basking opportunity on juvenile development and phenotypic characteristics have rarely been examined (but see Shine and Harlow, 1993).

Pregnancy confers many costs to a female lizard. These include physiological or metabolic costs (Beuchat and Vleck, 1990; DeMarco and Guillette, 1992), feeding difficulties (Schwarzkopf, 1996a), decreased mobility and increased predation risk (Shine, 1980; Sinervo, Hedges and Adolph, 1991; Schwarzkopf and Shine, 1992; Chapter 8 of this thesis). Presumably females act to reduce these costs, either by behavioural shifts (Bauwens and Thoen, 1981; Cooper *et al.*, 1990) or by decreasing gestation length (Beuchat, 1988; Schwarzkopf and Shine, 1991). Gestation length is generally decreased by maintaining body temperatures suitable for rapid embryonic development for longer periods and is generally achieved by increasing basking duration and frequency (Beuchat, 1988; Schwarzkopf and Shine, 1992; Daut and Andrews, 1993; Lecomte, Clobert and Massot, 1993).

Many authors have documented an increase in basking time by gravid lizards (Beuchat, 1986; 1988, Schwarzkopf and Shine, 1991). Increased basking reduces gestation length in at least one skink species, *Eulamprus tympanum* (Schwarzkopf and Shine, 1991), and it is likely to have similar effects in other shuttling heliotherms. The selective advantages to the mother in reducing the length of gestation are numerous, but the implications for the developing embryo and ultimately the effect on juvenile phenotype and fitness are less well documented.

Differences in the phenotype of newborns (especially size or condition) are ecologically important, especially when such differences can be related to differences in performance and growth (Brockelman, 1975). Size at birth affects neonatal growth, locomotor performance, and survivorship of many reptile species (e.g. Ferguson and Fox, 1984; Vleck, 1988; Jayne and Bennett, 1990; Sinervo, 1990b; Olsson, 1992; Sinervo *et al.*, 1992; Sinervo and Adolph, 1994). For example, larger offspring may grow more rapidly, pass quickly through size classes more vulnerable to predation, better survive poorer conditions, reach reproductive size more rapidly and catch food more effectively than smaller offspring. Miller (1993) argued, for example, that differences in birth size and performance of hatchling turtles may be ecologically important because larger animals may be able to move toward water from their nest sites more quickly than their smaller siblings.

Niveoscincus ocellatus is an ideal species in which to test the influence of gestation conditions on juvenile phenotype and fitness. Throughout its range it displays significant differences in life history characteristics and in timing of reproductive cycles (Chapters 3, 4, and 5). In the previous chapters I demonstrated that much of the variation in life history traits (including growth rates and size and age at maturity) could be attributed to proximate influences of the thermal environment, rather than genetic differences between populations. Juvenile size (length and weight) differ between the Orford and Central Plateau study populations, although the mechanism(s) responsible for these differences is not yet known. At my two study sites there are distinct differences in climate

(see Chapter 2); activity is restricted both on a daily and seasonal basis at the Central Plateau. Pregnant lizards from these sites may experience very different opportunities for basking and consequently the developing embryo is also likely to experience different conditions for development suggesting perhaps this is a proximate source of variation in juvenile phenotype.

In this study, the effect of basking opportunity provided to female lizards from the Orford study site (see Chapter 2) is investigated and four specific questions addressed:

1. does basking opportunity provided to females affect gestation length?
2. does gestation length affect female condition?
3. does gestation length affect juvenile phenotype?
4. does juvenile phenotype affect performance and/or growth rate?

7.2 Materials and methods

7.2.1 Gestation length

Female *N. ocellatus* were collected from the Orford study site between October 7 and 17, 1996. Mated mature and reproductively active females were identified in the field by the presence of obvious mating marks (Figure 3.1, Chapter 3). The lizards were taken to the laboratory where the presence of large follicles was assessed by palpation. Ovulation was confirmed in a small subsample (eight lizards) by dissection. The presence of ova within the oviducts and large corpora lutea confirmed recent ovulation.

Thirty four females were included in the experiment and these were randomly assigned to one of two treatments on 20 October 1996; 18 lizards were provided with 4 hour access to a radiant heat source and 16 lizards were provided with 10 hour access to a radiant heat source. All animals were maintained in those conditions until their young were born.

All lizards were maintained in the same room under the same light cycle of 14L:10D (see Chapter 1). Females were housed in pairs in plastic terraria (20 x 30 x 10 cm) and supplied with water *ad libitum* and food three times weekly (generally 2 mealworms per lizard on each occasion, with soft fruit supplied for variety). Each terrarium was positioned randomly within the experimental regime, and cages were re-positioned daily to minimise position effects (Figure 7.1).

Basking heat was supplied by a 25 W incandescent globe suspended approximately 8 cm above a basking surface. To further concentrate the heat and provide a greater thermal gradient the bulb was surrounded by a small metal can. A small shallow overturned terracotta pot was used as the basking surface. A thermal gradient from 30-35 °C at the basking surface and approximately 18 °C in the rest of the cage allowed lizards to thermoregulate while the basking light was on. The ambient temperature fell to approximately 10 °C at night. Each cage was supplied with adequate cover which the lizards could use when they were not basking.

Females were held until parturition and weighed to calculate relative clutch mass (RCM), which is defined as in Chapter 4 as:

RCM = total mass of newborns (mg)/mass of female after birth (mg).

Following parturition, a subsample of females was killed and fat bodies removed and weighed to assess maternal condition. A second measure of female body condition (mg/mm) was calculated as:

condition (mg/mm) = female body mass (mg)/female SVL (mm).

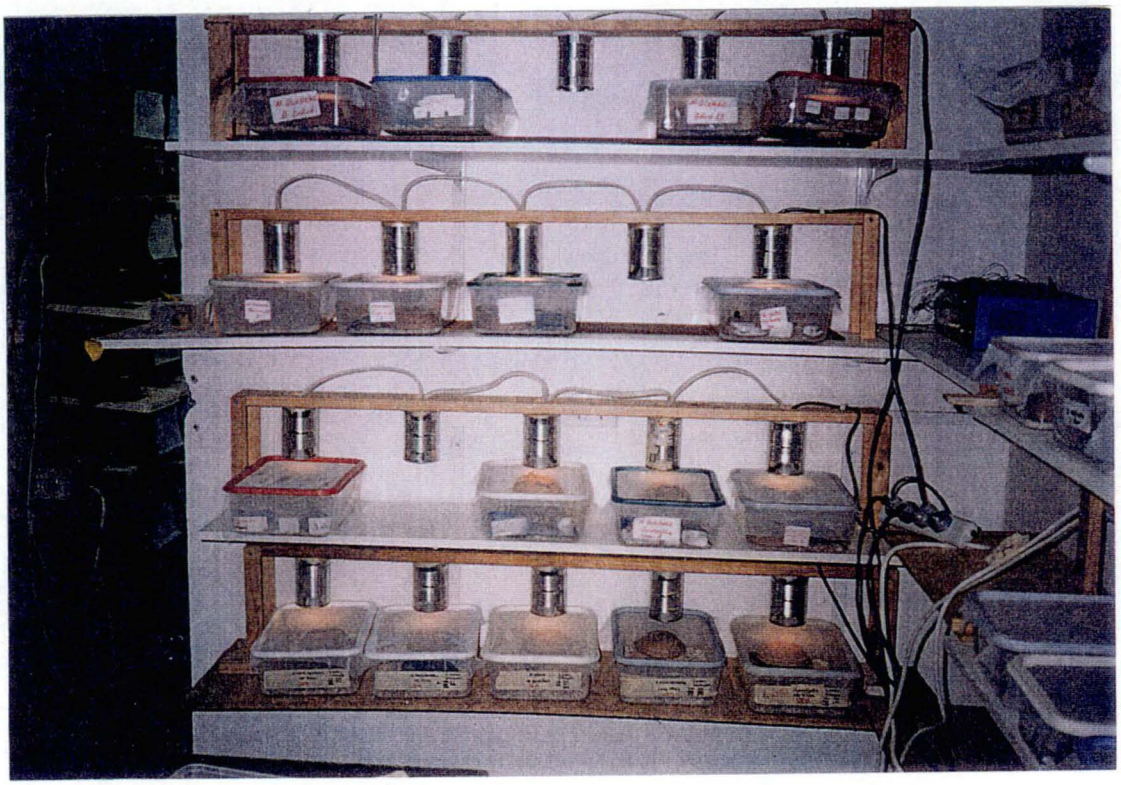

Figure 7.1. Experimental arrangement showing cages with radiant heat sources provided to allow *Niveoscincus ocellatus* females to bask.

7.2.2 Birth and juvenile details

At birth, newborns were measured, sexed and their performance tested (all as described in Chapter 6).

7.2.3 Juvenile growth rate

Juvenile lizards were maintained in plastic terraria (20 cm x 30 cm x 10 cm) in the same conditions described for the females during gestation. Basking conditions (25 W globe as for females above) were available for

12 hours each day. The cages were held in an air conditioned room, on two shelves. The position of each cage was randomly re-assigned on the shelves each day in case there were minor differences between cage positions. Up to ten lizards were maintained in each cage. Observations showed that there was no competition for basking resources or food, although occasionally two lizards would fight over a particular food item (despite excess food in nearby food dishes). Each cage was supplied with water *ad libitum*, and food was supplied daily. Very small mealworms were given daily and in addition crushed banana mixed with multivitamins (Wombaroo Herpatavite Reptile Supplement and vitamin D3) was given approximately three times weekly. All cages were given the same food on the same days, and the food was generally in excess.

In order to minimise disturbance all lizards were measured on the same day which resulted in growth periods of between 41-91 days (because lizard birth dates differed). Growth rates were calculated as for Chapter 6.

7.3 Results

7.3.1 Female characteristics

Snout vent length of the 34 females ranged from 57 mm to 68 mm (mean 63.6 mm), with the predicted clutch (determined by palpation) ranging from 1 to 4 (mean 2.48). In all cases the predicted clutch based on palpation was the same as the actual clutch obtained from birth details. The lizards were randomly assigned to their experimental regimes and there was no significant difference in sizes or weights between the groups ($F_{1,32} = 0.723$; $P > 0.1$; $F_{1,32} = 0.085$; $P > 0.1$ respectively).

The majority of females settled well into captivity. There were no observed agonistic behaviours between pairs of lizards and when they were examined no bite marks on animals were found. In fact in many cases the lizards were observed either sharing the basking surface supplied or sheltering beneath the same cover, despite individual shelter

being available. Overt basking typically began when the overhead light was switched on, but lizards spent most of the day basking covertly beneath the basking surface. All lizards fed regularly, with excess food generally available. The lizards with access to 4 hours heat generally ate less than those given access to 10 hours heat. Thermoregulatory behaviour was constrained only by the potential basking time, not by available basking space.

7.3.2 Gestation length and female characteristics

All of the lizards assigned to the 4 hour basking regime gave birth, while in the 10 hour group, two females aborted premature babies (approximately stage 37-38 (Dufaure and Hubert, 1961) and no birth was recorded from a third female. Of the eight lizards that were dissected in early October prior to the beginning of the experiment, six had ovulated and two had large follicles that were judged to be close to ovulation. Thus, in the Orford population gestation began between late September and early October, and by the beginning of the experiment (17 October) it is assumed that all females had ovulated. For the purposes of comparison it is assumed that all females had ovulated by the beginning of October and gestation lengths are calculated from October 1.

Females that were provided with 10 hours access to basking gave birth between January 10 and 29 (gestation length 102-121 days) and those provided with 4 hours access to basking gave birth between January 29 and March 19 (gestation length 121-170 days) (see Figure 7.2). The same population gave birth in the wild between 1 January and 3 February (gestation length 92-126 days). Table 7.1 provides details on the female characteristics, including size, weight and condition following gestation. Chapters 4 and 6 provide further details on the offspring size and weight for the wild population.

Table 7.1. Female *Niveoscincus ocellatus* characteristics. All values are means ± standard error and the sample size is indicated in parentheses. (* denotes significance, $P < 0.05$).

trait	10 hour basking	4 hour basking	sig
snout vent length (mm)	63.1 ± 0.87 (15)	64.1 ± 0.81 (18)	ns
initial mass (g)	4.4 ± 0.21 (15)	4.5 ± 0.23 (18)	ns
clutch size (number of young)	2.3 ± 0.21 (15)	2.7 ± 0.23 (18)	ns
gestation length (days)	105.3 ± 2.26 (13)	140.6 ± 4.08	*
gestation length (range)	102 -121 (13)	121 -170 (18)	*
female condition (mg/mm)	79.1 ± 3.24 (13)	75.6 ± 2.36 (18)	ns
female fat (mg fat bodies)	322.5 ± 46.85 (11)	282.6 ± 29.63 (15)	ns
RCM (clutch mass/female mass)	0.23± 0.022 (13)	0.26 ± 0.018 (18)	ns

In all cases the predicted clutch was the same as the actual clutch size obtained from birth data and thus palpation is a reliable predictor of final clutch size indicating that there is little or no loss after ovulation. Similarly, Jones, Wapstra and Swain (1997) found that loss of vitellogenic follicles was less than 0.3 % in this species. There were no differences in the predicted clutch size between groups ($F_{1,31} = 1.628$; $P > 0.1$). Despite significant differences in juvenile phenotype (Table 7.2), there were no significant difference in RCM ($F_{1,29} = 0.860$; $P > 0.1$), female condition ($F_{1,29} = 0.841$; $P > 0.1$), or mass of abdominal fat bodies in females ($F_{1,24} = 0.569$; $P > 0.1$).

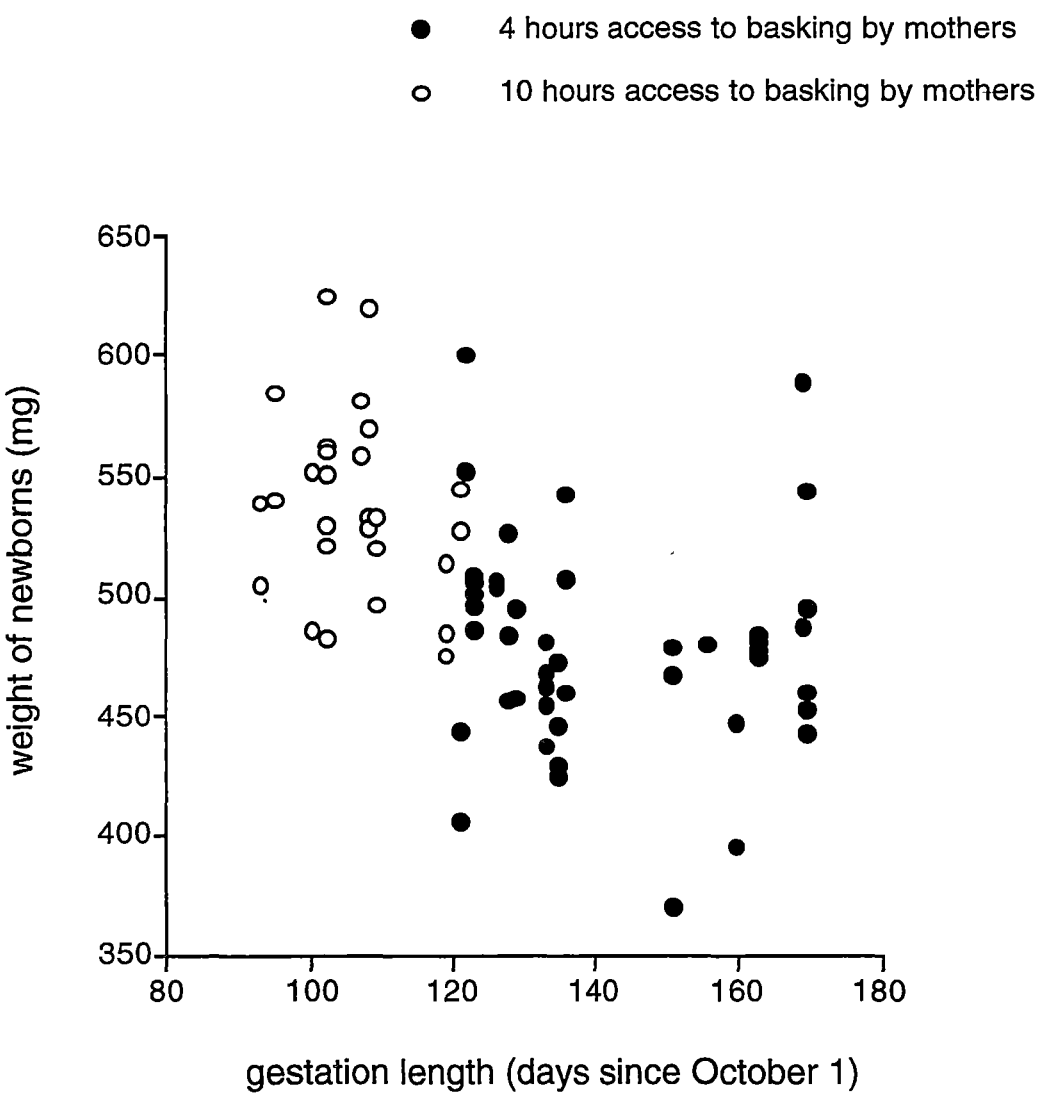

Figure 7.2. Gestation length (days) and weight of newborn *Niveoscincus ocellatus* from mothers provided with either 4 or 10 hours access to basking opportunities. October 1 is assumed to represent to beginning of pregnancy for mothers and they were placed in their experimental regimes on October 17.

7.3.3 Juvenile details

Table 7.2. Details of juvenile *Niveoscincus ocellatus* phenotype, performance and growth. All values are means ± standard errors with sample size indicated in parentheses. (* denotes significance, $P < 0.05$).

juvenile character	10 hour	4 hour	sig
snout vent length (± 0.05 mm)	29.8 ± 0.19 (27)	29.2 ± 0.13 (45)	*
total length (± 0.1 mm)	68.9 ± 0.48 (27)	66.4 ± 0.45 (44)	*
proportion (snout-vent/total)	2.31 ± 0.009 (27)	2.27 ± 0.010 (44)	*
weight (± 0.1 mg)	537.9 ± 7.34 (27)	478.3 ± 6.50 (46)	*
condition (mg/mm)	18.0 ± 0.21 (27)	16.4 ± 0.18 (45)	*
growth rate (mm/day)	0.06 ± 0.005 (25)	0.04 ± 0.005 (36)	*
sprint speed at birth (m/sec)	0.96 ± 0.073 (21)	0.94 ± 0.056 (36)	ns
sex ratio (males: females)	9:16	30:14	*
survival (% during growth period)	93	80	

Juveniles born to females provided access to 10 hours basking were significantly larger at birth (snout vent length: $F_{1,70} = 9.768$, $P < 0.01$; total length: $F_{1,69} = 13.52$, $P < 0.01$; weight: $F_{1,71} = 34.197$, $P < 0.01$). The juveniles also differed in allometry; newborns from the 10 hour group had significantly longer tails than those from the 4 hour group ($F_{1,69} = 4.806$, $P < 0.05$) and they were also in better condition ($F_{1,70} = 32.438$, $P < 0.01$). The growth rates also differed between groups ($F_{1,59} = 8.471$, $P < 0.01$). There was no significant difference in sprint speeds of the two groups ($F_{1,55} = 0.031$, $P > 0.1$). The ratio of male to female offspring did not deviate from a 1:1 ratio in the 10 group ($P > 0.1$), but there were significantly more males than females in the 4 hour group (Chi-square

= 5.818; $P < 0.05$). The ratio of males to females also differed significantly between treatments (Chi-square = 5.145; $P < 0.05$).

Sprint speed appeared to be a poor measure of juvenile fitness. There was no relationship between sprint speed at birth and length and weight at birth or between sprint speed and subsequent growth rate ($P > 0.05$ in all cases). Although there was no difference between sprint speed and post-natal growth rate, growth rate was related to other measures of juvenile phenotype at birth (independent of the maternal gestation conditions) including snout vent length ($F_{1,59} = 5.013$, $P < 0.05$); weight ($F_{1,59} = 9.273$, $P < 0.01$) and condition ($F_{1,59} = 7.064$, $P < 0.05$).

7.4 Discussion

7.4.1 Gestation length and female condition

Gestation length in *N. ocellatus* depends on the basking behaviour of the mother. This result is not surprising and has been identified in other species (Beuchat, 1986; Schwarzkopf and Shine, 1991). The rate of development of reptilian embryos is temperature dependent, and higher temperatures within the normal range accelerate embryonic development (Castilla and Swallow, 1996). Reduced development times in reptilian embryos exposed to elevated temperatures are common in either constant conditions (e.g. Van Damme *et al.*, 1992) or under more naturally cycling thermal conditions (Castilla and Swallow, 1996). Presumably, in my study, opportunity for basking allowed the females to maintain a higher body temperature for longer periods, resulting in more rapid embryogenesis.

In this study, females provided with 10 hours of potential basking time had parturition dates that overlapped with parturition dates for the Orford population under natural conditions at the study site (Chapter 3). Females provided with only 4 hours of access to basking did not begin to give birth until the other experimental group had finished. However, parturition in the 4 hour group did show some overlap with the natural

population at Orford, although most females gave birth on dates that are more typical of the colder Central Plateau site. This suggests that the experimental design was a reasonable test of differences in access to basking that may be encountered at the climatic extremes occupied by *N. ocellatus*.

Despite the large difference in mean gestation length between the two treatments, in both experimental groups the majority of females carried viable embryos to full term and in the group that displayed lengthened gestation all females produced viable clutches. This has important implications for species that occupy wide geographic ranges, such as *N. ocellatus*. Over a wide geographic or climatic range, it is likely that there will be considerable variation in weather suitable for activity, and thus basking duration may be affected. At sites experiencing colder conditions, gestation may be lengthened. Although development within the female provides a mechanism for reducing development time and buffering embryos against the deleterious effects of incubation at low temperatures (DeMarco, 1992), the developing embryo is reliant on the body temperature of the female for development.

An ability to produce viable clutches despite delays may well be an important adaptation allowing *N. ocellatus* to occupy a wide geographic and climatic range. Delayed parturition in response to cold weather has been observed in other viviparous species (Beuchat, 1989). Thus in populations occupying cold environments or in colder seasons there may be strong selective pressures for females to bask (ie. maintain body temperatures at or near optimum) as often as possible, whereas this may be less important in populations occupying warmer climates, or after females have given birth. The body temperatures selected by a female are likely to be a reflection of both the optimum temperature for her and for embryonic development, but these may be constrained by either the environment or other ecological constraints (Tosini and Avery, 1996).

In natural conditions in which basking opportunities are reduced even further than the equivalent of the 4 hour basking regime in this experiment, it is unlikely that parturition could occur within the same

season. Interestingly, species within the genus *Niveoscincus* that occur at higher altitudes than the limit of the range of widespread species such as *N. ocellatus* and *N. metallicus*, display an unusual biennial reproductive cycle (Swain, 1972; Rawlinson, 1975; Hutchinson, Robertson and Rawlinson, 1989; Olsson pers. comm.). In the alpine specialist species, the northern snow skink *N. greeni* and the southern snow skink, *N. microlepidotus*, some females overwinter developing embryos, with parturition occurring the following spring after an extended gestation period. The significance to the embryos of this prolonged gestation is not known. This ability to overwinter viable embryos also occurs in the gecko *Hoplodactylus maculatus* and may well represent an important adaptation allowing these species to occupy alpine regions (Cree and Guillette, 1995).

In my experiment the length of gestation did not affect the condition of the females. Females from both experimental groups had large fat bodies, and were similar in weight for their size. Pregnancy typically confers energetic and other costs to lizards (see discussion in Chapter 3); however two factors may explain the similarity in the condition of the females in this experiment. Firstly, conditions in the terraria in the laboratory are unlikely to reflect conditions typically experienced in field situations. For example there was generally excess food requiring little capture effort. Secondly, there is some doubt that late stages of pregnancy do indeed confer a substantial energetic cost to the female *N. ocellatus*. In Chapter 3, I demonstrated that fat reserves actually increase in the late stages of gestation at both study sites.

7.4.2 Offspring characteristics

7.4.2.1 Offspring phenotype

In this study, access to basking by female *N. ocellatus* markedly affected the phenotype of their offspring. Similarly, Shine and Harlow (1993) found that maternal access to basking affected offspring phenotype in the viviparous skink, *Eulamprus heatwolei*. In my study, females that were

given limited basking opportunities gave birth to significantly smaller and lighter offspring than those given more basking time. The condition of the offspring was affected also, with larger offspring in better condition being born to females with greater access to basking opportunities. Although many studies have demonstrated that the phenotype of hatchlings may be modified by incubation conditions in a variety of reptiles (e.g. Webb, Choquenot, and Whitehead, 1986; Phillips *et al.*, 1990; Van Damme *et al.*, 1992; Miller, 1993; Shine and Harlow, 1993; Webb and Manolis, 1993; Castilla and Swallow, 1996; Mathies and Andrews, 1997), including skinks (Shine, 1995; Shine and Harlow, 1996), few studies have demonstrated that the maternal environment of viviparous species may have similar effects (Shine and Harlow, 1993; Mathies and Andrews, 1997). Clearly, my study suggests that behavioural decisions (especially thermoregulatory behaviour) by female lizards have the potential to modify the phenotype of their young.

Several mechanisms may act to produce intraspecific variation in the size of offspring at birth, most of which act prior to ovulation. For example, there may be differences in the amount of yolk reserves provided, either through differences in availability of energy or in selection for different sized young (e.g. Nussbaum, 1981; Sinervo, 1990b; Sinervo and Licht, 1991b). However, the mechanism(s) for producing different sized young after ovulation in viviparous skinks, such as *N. ocellatus*, remains poorly understood. Shine and Harlow (1993) suggested that incubation temperatures might affect neonatal characteristics either by direct action on the embryo or indirectly through the mother's response to lowered basking opportunities. They further argued that an indirect response is unlikely because incubation conditions similarly affect hatchling characteristics of oviparous species, including skinks (Burger, 1989; 1990; Shine, 1995; Shine and Harlow, 1996).

The yolked egg represents the majority of the female input into the developing embryo, although the transfer of organic and inorganic compounds has been demonstrated for a variety of lizard species (e.g. Thompson, 1981; 1982; Stewart and Castilla, 1984; Yaron, 1985;

Stewart and Thompson, 1993; 1994; Swain and Jones, 1997) and may be quite common in species with relatively complicated placentae (Swain and Jones, 1997). Swain and Jones (1997) suggested that female *N. metallicus* may have the capacity to modify the placental input according to environmental circumstances and embryonic needs.

These authors (Swain and Jones, 1997) suggested that the ability to supplement lecithotrophic nutrition (yolk) with organic matrotrophy after ovulation might provide a means of supplementing the yolk reserves, laid down in the previous autumn, with reserves available later during gestation in the following spring. This mechanism may result in larger offspring or enable birth to be deferred until conditions are optimum for birth or neonate survival, without endangering offspring fitness, even when all yolk reserves have been consumed. However, in my study there is little evidence to support this, as a delay in parturition (as might be brought about by poor weather) in fact reduced offspring fitness. If the placenta represents a major avenue for supplemental female input into the young, then one might expect that, as gestation length increases, newborns may remain a similar size (or be larger) to newborns born after a more rapid gestation.

Incubation temperatures affect the degree to which yolk is converted to body tissues (Shine and Harlow, 1993), so that at lower temperatures embryos hatch with a greater yolk free mass, but less residual yolk. Shine and Harlow (1993) argued that this may explain the difference in body length and shape in neonatal *Eulamprus heatwolei* exposed to different thermal treatments during embryonic development. However, they did not detect any difference in body mass of neonates between treatments. Perhaps the simplest explanation for the observed differences in body mass between *N. ocellatus* offspring from different treatments is that, as gestation length is increased beyond normal lengths, the developing young are required to divert energy stores (supplied as yolk), including nutrients and lipid reserves, towards cellular maintenance, rather than growth. This would explain the difference in offspring size, both in length and mass. The condition of the neonates at birth supports this argument. Condition at birth is likely to be of great importance to

neonates, especially early in life when stored fat reserves may be used for growth.

The effect of external conditions on embryonic development may vary throughout the development period. For example, in crocodiles, hatchling characteristics are more influenced by temperature during the last third of development (embryo growth phase) than during the first two-thirds of development (Congdon, Fischer and Gatten, 1995). Certainly an important future direction of this type of work with viviparous species should be to describe in more detail the period or periods during gestation in which offspring characteristics are most susceptible to modification by external factors, such as female basking behaviour or female condition and energy availability.

The mechanism affecting offspring phenotype in reptiles from different incubation or gestation conditions may vary across groups. Reduced development time produces smaller hatchlings in some oviparous lizards (Beuchat, 1988; Phillips *et al.*, 1990; Van Damme *et al.*, 1992), viviparous lizards (Beuchat, 1988; Mathies and Andrews, 1997), crocodiles (Webb, Choquenot and Whitehead, 1986; Webb and Manolis, 1993), or has no effect (other oviparous lizards) (Castilla and Swallow, 1996) and produces larger young in turtles (Campos, 1993; De Souza and Vogt, 1994) and other oviparous lizards (Shine and Harlow, 1996). However, much of this available evidence is based on laboratory trials of egg laying species, where eggs are generally exposed to different regimes of constant conditions (Castilla and Swallow, 1996) and it is only more recently that the possible importance of more "natural" conditions for development have been investigated.

Constant conditions for either gestation or incubation are "rarely, if ever", encountered in nature, and the physiological potential revealed in laboratory experiments may not be realised in the field (Cagle *et al.*, 1993). However, weather characteristics may affect offspring phenotype in the field (Olsson and Shine, 1997). Female sand lizards, *Lacerta agilis*, exposed to a higher proportion of cloudy days prior to laying produced hatchlings that were smaller. Although this may be related to the energy available to

female lizards and the resultant energy devoted to reproduction, the effect of weather conditions on female basking behaviour, and therefore on embryo development, should not be ignored.

Interestingly, there were differences in the sex ratios of newborns from the two treatments. More male offspring were born after a prolonged gestation period. A similar result was obtained in the natural population at Orford, where an offspring born later in the season was more likely to be male (Chapters 4 and 6); however, until further work is conducted on this phenomenon the mechanism by which the sex of offspring of *N. ocellatus* may be affected by gestation conditions remains unclear.

7.4.2.2 Juvenile performance and growth rate

Growth rates of newborns were significantly affected by the basking regime of their mothers. Offspring born to females that were provided good basking opportunities grew more rapidly under standard laboratory conditions than offspring born to females provided with poor basking opportunities. In this study, the offspring that were born first (10 hour group) were also born in better condition. It is likely that their condition allowed them to devote a greater amount of energy to growth, with consequent faster growth than neonates born in poorer condition. The influence of size on fitness depends on larger young maintaining their size, speed, or other competitive advantages over smaller individuals (Miller, 1993). In this study, there are significant differences in newborn size between treatments, and these become increasingly marked after a period of growth. These differences may be ecologically relevant because offspring that are born first are bigger at birth, have a longer growing season, and grow more rapidly.

These factors presumably result in the young that are born first maintaining or even increasing their competitive advantage over young that are born later. The selective advantages of rapid growth are numerous and are discussed in more detail in Chapter 6. Growth rates of juveniles are often related to measures of success as adults (Doody, 1996).

For example, more rapid juvenile growth, through its effect on juvenile size, may affect a number of fitness related performances including sprint speed (e.g. Sinervo and Adolph, 1989), acquisition of territories (e.g. Fox, 1978; Ferguson, Brown and DeMarco, 1982; Stamps, 1983; 1988), dispersal distance (Doughty and Sinervo, 1994; Doughty, Sinervo and Burghardt, 1994), predator escape (Dunham, 1978) and survival (Ferguson and Fox, 1984; Sinervo *et al.*, 1992; Sinervo and Adolph, 1994). Furthermore, growth rate of juveniles has been shown to affect other important life history traits (see Chapter 6 and references therein).

The factors that influence growth rates are numerous and well studied (see Chapter 6). However, the developmental environment of the embryo has been recognised as a source of variation in growth rate only relatively recently (Overall, 1994) and has been investigated only twice in viviparous species (Shine and Harlow, 1993; Mathies and Andrews, 1997). Sinervo and Adolph (1994) suggested that growth rates of hatchling lizards are probably much more sensitive to current thermal environments than to temperatures experienced during incubation as they did not detect differences in growth rates between juveniles born from incubation treatments at different temperatures.

Although this study was able to demonstrate significant differences in offspring morphology at birth and subsequent differences in growth rates, sprint speed of newborns was a poor predictor of juvenile growth rate. Furthermore, sprint speed was not related to measures of offspring phenotype, such as length or weight, despite these measures being significantly related to subsequent growth rate. Although it is now widely accepted that sprint speed represents a whole body measure of performance that may be related to some measures of fitness (ie. it is often related to other measures of fitness such as predator escape and feeding opportunities), it appears that in this study this is not the case. It may be argued that the only true measure of fitness differences between different offspring phenotypes is a comparison of survival or growth rate in a natural environment where realistic selective pressures exist.

It has long been accepted that there are benefits to a female viviparous lizard in producing young as quickly as possible in any given environment and evidence of increased basking and more careful thermoregulation by gravid lizards supports this view (e.g. Beuchat, 1986; 1988; Charland and Gregory, 1990; Schwarzkopf and Shine, 1991; Daut and Andrews, 1993; Lecomte, Clobert and Massot, 1993; Tosini and Avery, 1996; Mathies and Andrews, 1997). Some of the previously documented benefits of a shorter gestation for the female include: reducing the energetic cost to the female (Birchard *et al.*, 1984; Beuchat and Vleck, 1990; DeMarco and Guillette, 1992; DeMarco, 1993), reducing the loss of feeding opportunities that are often associated with pregnancy (Schwarzkopf, 1996a), and decreasing the time during which females may be more susceptible to predation (Bauwens and Thoen, 1981; Shine, 1980; Cooper *et al.*, 1990; see also Chapter 8 of this thesis).

In addition to the above benefits , this study (and Shine and Harlow, 1993) suggests that there may be other benefits to the female in reducing gestation length. A female's fitness is ultimately determined by the fitness and success of her offspring; females that modify their basking behaviour and produce young more rapidly produces larger, fitter offspring that are more likely to survive. Furthermore, these offspring also then have a longer potential growing season. There is thus the potential for selection to act on factors influencing gestation length such as the timing of the gestation period (see Chapter 3) or maternal basking behaviour (this chapter). The phenotype of offspring results from of a complex interaction of a number of factors, including genetic make-up (maternal and paternal; e.g. Vleck, 1988; Olsson *et al.*, 1996), energy allocated to each progeny (e.g. Sinervo, 1990b; Sinervo and Licht, 1991a; 1991b), selection of incubation environment in oviparous species (Congdon, Fischer and Gatten, 1995; Shine and Harlow, 1996) or maternal behaviour during pregnancy (this study and Shine and Harlow, 1993).

Differences in life history traits between populations of geographically widespread species are often explained in terms of genetic divergence, presumably resulting from different selective pressures present in the populations. However, more recently variation in life history traits has

been demonstrated to result from phenotypically plastic responses to environmental variables (e.g. Adolph and Porter, 1993; 1996; Rohr, 1997; Chapter 6 of this thesis). The results from the present study demonstrate that offspring phenotype and juvenile growth are phenotypically plastic and can be modified by manipulating maternal basking behaviour. These data again emphasise the importance of proximate influences (particularly the thermal environment) on the life history traits of *N. ocellatus*, and invite caution in interpreting geographic variance in traits in terms of genetic divergence.

The results of the present investigation suggest a mechanism to explain annual variation in juvenile phenotype in natural populations. Annual differences in weather characteristics are likely to influence lizard activity, and more specifically basking opportunities for gravid females. The amount of basking opportunities then has the potential to affect gestation length and juvenile phenotype. For example, in the 1995/1996 season offspring from the Orford study site were smaller (length and weight) than in other years. During this season the weather was unusually cool and wet (Chapter 2); this resulted in reduced basking opportunities for females and parturition was delayed.

It was also the intention of the present investigation to suggest a possible proximate mechanism that may lead to the consistent difference in offspring size between the two study sites. The experiment reported in this chapter indicates that, all else being equal between the populations, offspring from the Central Plateau study site should be smaller because of the reduced basking opportunities at this site. This is completely contrary, of course, to the trend actually observed; at the Central Plateau study site neonates were consistently larger in all years (1994-1997)(Chapter 4 and 6). In Chapter 3, I suggested that one mechanism that may reduce the potential influences of a colder environment on gestation length (gestation length is identical in both populations) and juvenile phenotype is through a shift in the period during which gestation actually occurs. At the Central Plateau gestation proceeds through the warmest months (November, December and January) while at the Orford site it occurs earlier in the season (Chapters 2 and 3). Certainly other

factors differ within and between populations and these also contribute to observed differences in offspring phenotype (see Chapter 4); they include genetic differences (e.g. selection for different sized young), food resources, female allocation to reproduction generally or the investment per offspring, physiological differences, and behaviour. It is the interplay of all these factors, and an environmentally mediated response, that ultimately results in the observed phenotype of offspring.

Chapter 8.

Cost of reproduction in *Niveoscincus ocellatus*: a reduction in sprint speed by gravid lizards

8.1 Introduction

Costs are believed to be an important determinant of the evolution of reproductive effort in squamate reptiles (Shine, 1980; Vitt and Price 1982; Shine and Schwarzkopf, 1992; Schwarzkopf, 1993; 1994). Reproductive costs associated with viviparity can be broken down into two major categories: physiological costs and ecological costs. Physiological costs include the metabolic demands of pregnancy, depletion of energy resources and loss of condition (e.g. Birchard *et al.*, 1984; Beuchat and Vleck, 1990; DeMarco and Guillette, 1992; DeMarco, 1993). Ecological costs include increased vulnerability to predators, lost reproductive opportunities, trade-offs in time activity budgets, loss in growth, and reduced feeding opportunities (e.g. Beuchat, 1986; Brodie, 1989; Schwarzkopf and Shine, 1992; Schwarzkopf, 1992; 1993; 1996a; Landwer, 1994). Although some of these costs are not unique to viviparous species, the extended period of gestation displayed by viviparous squamates may make these costs significantly more important (Shine, 1980; Seigel and Fitch, 1984).

Recently, there have been several studies on the influence of reproduction on performance and behaviour of female reptiles (e.g. Shine, 1980; Bauwens and Thoen, 1981; Seigel, Huggins and Ford, 1987; Brodie, 1989; Cooper *et al.*, 1990; Schwarzkopf and Shine, 1992). These studies have concentrated on the effects of the physical burden of pregnancy on female performance. One measure of the physical burden imposed on gravid females is the relative clutch mass (RCM), which may be defined as the ratio of the clutch mass to the female body mass at parturition (Vitt and Congdon, 1978; Shine, 1980; Sinervo, Hedges and

Adolph, 1991). It has been argued that RCM represents a distinct life history trait of squamate reptiles that is optimised via differential mortality of gravid females (e.g. Vitt and Congdon, 1978; Vitt, 1981; Vitt and Price, 1982; Seigel and Fitch, 1984). The ecological correlates of variation in RCM among species are well documented. Widely foraging lizards or active foragers tend to travel relatively long distances to escape predation and have relatively low RCMs, while sit-and-wait predators tend to move relatively shorter distances, to rely on crypsis rather than speed and have a relatively higher RCM (Vitt and Congdon, 1978; Huey and Pianka, 1981; Vitt and Price, 1982; Magnusson *et al.*, 1985).

Locomotor impairment has two important ecological costs associated with it: an increase in predation risk (e.g. Shine, 1980; Christian and Tracy, 1981; Madsen, 1987; Cooper *et al.*, 1990) and a decrease in feeding ability (Shine, 1980; Schwarzkopf, 1996a). A decrease in running speed is likely to incur an even greater burden on the female if the species is an active forager or if running is the usual method of escape from predators. Among actively foraging lizard species, those with the greatest decrements in sprint speed are likely to suffer a greater risk of predation (Cooper *et al.*, 1990). Cooper *et al.* (1990) argue that for species with similar body proportions within a lineage, those with a high RCM should be most affected. Similarly, within species those individuals with a larger clutch (either actual mass or RCM) may be expected to show the greatest decrements in performance.

A negative relationship between clutch mass and performance abilities (e.g. sprinting ability) may be an important selective pressure shaping the evolution of reproductive effort (Vitt and Price, 1982). As predicted, the degree of locomotor impairment increases with an increase in RCM in some reptile species (Shine, 1980; Van Damme, Bauwens and Verheyen, 1989), but not in others (Brodie, 1989; Sinervo, Hedges and Adolph, 1991). At an individual or population level, the RCM would presumably be a result of selective pressures acting on survival behaviours, including predator escape and foraging behaviour, as well as phylogenetic constraints (Vitt and Price, 1982). Relationships between energetic investment in reproduction, foraging mode, and escape tactics may have

strongly influenced squamate life histories (Vitt and Congdon, 1978; Dunham, Miles and Reznick, 1988).

Few studies have examined the decrease in mobility in small lizards as a result of pregnancy and related any decrease in mobility to the level of reproductive effort. In this study I investigated the effect of pregnancy on sprint speed for the two populations of *N. ocellatus* and further investigated whether any decrease in sprint speed was related to reproductive investment within each population. *Niveoscincus ocellatus* is an active, searching type forager (Wapstra and Swain, 1996) that relies on flight for predator escape (pers. obs.). Female *N. ocellatus* must carry the increased mass imposed by their offspring for most of the activity season, as they are gravid from October to January/February (Chapter 3).

8.2 Methods

Gravid *N. ocellatus* were captured from the Orford and Central Plateau study sites in October and November, 1995 at the onset of pregnancy and maintained in the laboratory under standard conditions (see Chapter 2) until after parturition. Each lizard was measured (± 0.1 mm) and given a unique toe clip for permanent identification. Toe clipping does not affect sprint speed in lizards (Huey, Overall, and Newman, 1990; Dodd, 1993). Recent tail breaks can reduce the running speeds of some lizards (Ballinger Neitfeldt and Krupa, 1979; Brown, Taylor and Gist, 1995), so only lizards with complete or completely regenerated tails were used.

The performance of a total of 129 lizards was tested. Two tests were conducted on each lizard. Lizards were sprinted approximately two weeks prior to parturition, and again two weeks after parturition. Non-gravid females (females that had previously given birth in the laboratory) were sprinted on both occasions as controls. Each lizard was weighed immediately preceding the trial. Sprint speed for each lizard is compared between gravid and non-gravid states and further related to the reproductive burden that the female was carrying. Reproductive burden

is defined as the difference in the mass of the female at the two trials. Although the difference in mass between trials does not necessarily represent RCM (RCM should be measured immediately after parturition), it does provide a reasonable measure of the physical weight burden that the female carried toward the end of pregnancy.

Each lizard was placed in a small sealed chamber immersed in a water bath held at 28 ± 0.1 °C for a minimum of 20 minutes. Each lizard was then "encouraged" to sprint along a "racetrack" (heated to 28 ± 0.1°C), two metre in length (10 cm wide), by gentle prodding from behind with an artist's paintbrush. The temperature of 28 ° C is close to the body temperature maintained in the field (unpublished data), to that at which sprint speed is maximised (Melville, pers. comm.), and the temperature at which other whole body processes are maximised (Wapstra, 1993). The time taken was recorded by three infrared lightbeams held 1 metre apart and linked to a Macintosh computer. Each run resulted in two times (one for each of the 1 metre sections). Lizards were sprinted down the track twice in rapid succession and the fastest of the four times chosen as the best representative of maximum sprint speed. Each run was given a rating, where 1 represented a good run, 2 an acceptable run, and 3 a poor run. A run was rated as "good" when the lizard needed little encouragement to run down the track and there was no stopping for the length of the track; an "acceptable" run had some slowing and the lizard needed further encouragement to run down the track, and "poor" runs resulted when the lizard either refused to run, stopped, or ran the wrong way. For the purposes of analyses any run that was given a rating of 3 was excluded. The ratings were independent of the actual sprint time.

8.3 Results

In total 129 lizards were sprinted, and of these 86 sprinted satisfactorily on both trials (Table 8.1).

Table 8.1. Number of female *Niveoscincus ocellatus* lizards used in successful sprint trials. Gravid lizards were gravid in the first trial and postpartum in the second trial. Control lizards were non-gravid females.

	Orford	Central Plateau
gravid	30	31
control	7	18

The SVL of the non-gravid control females did not differ from that of the pregnant lizards from the same site ($P > 0.5$ in both cases) but the sizes of lizards did differ between the sites. Lizards from the Central Plateau (CP) study site were significantly larger (mean ± SE = 73.8 ± 0.75 mm) than those from the Orford study site (mean ± SE = 62.3 ± 0.46 mm)($F_{1,84}$ = 146.32; $P < 0.001$). The Central Plateau females were also significantly heavier than their counterparts from Orford (Figure 8.1). The burden carried by the females from the two sites differed also, both in absolute weight (CP mean ± SE = 2686 ± 196.9 mg; Orford mean ± SE = 1307 ± 104.2 mg; $F_{1,59}$= 37.58; $P < 0.001$) and relative weight (relative burden = change in weight/female post-partum weight; see Shine, 1980 and Sinervo, Hedges, and Adolph, 1991 for discussion of the use of measures of RCM) (CP mean ± SE = 0.38 ± 0.028 mg/mm; Orford mean ± SE = 0.28 ± 0.023 mg/mm; $F_{1,59}$= 8.648; $P < 0.01$). The control lizards from the Central Plateau did not change weight in the time between trials while the Orford population increased in weight slightly (P = 0.018).

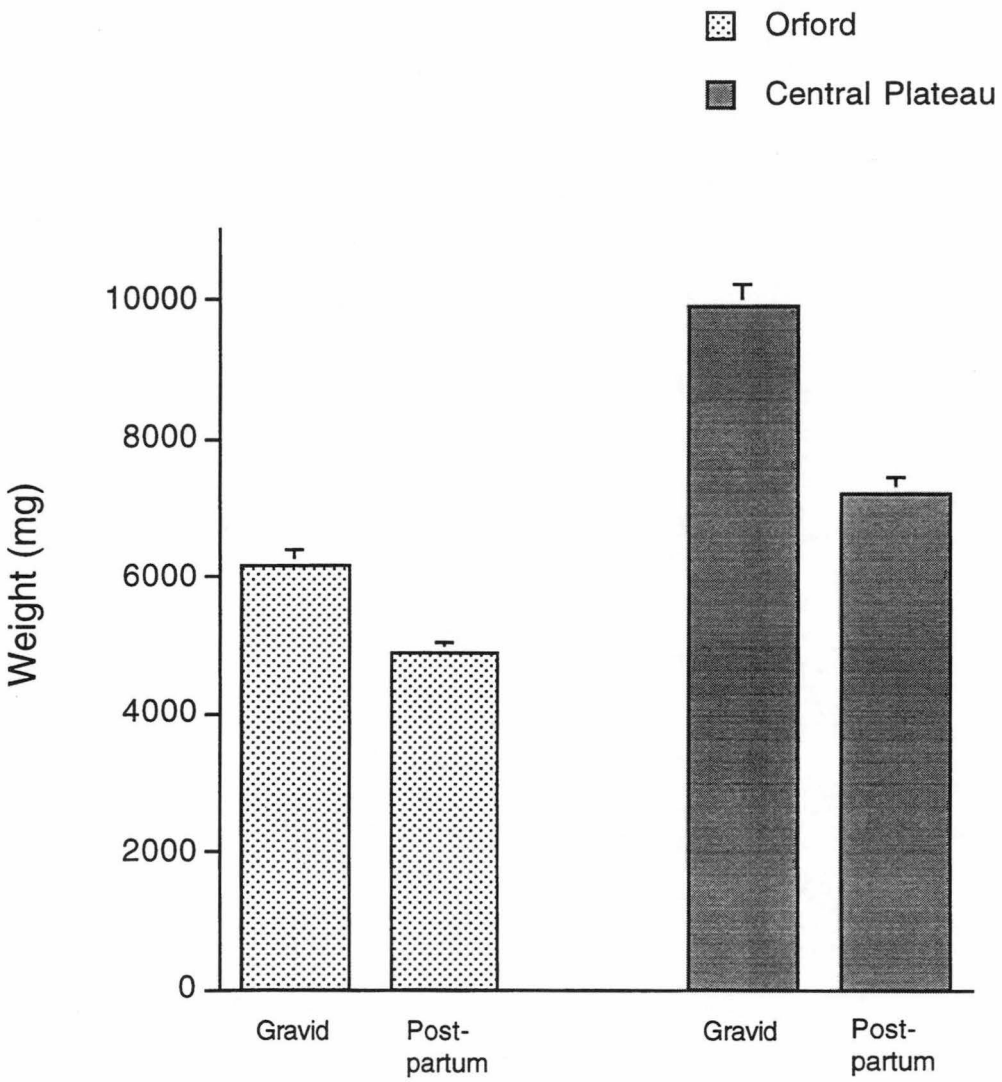

Figure 8.1. Change in weight of female *Niveoscincus ocellatus* at Orford and the Central plateau in gravid and postpartum states (mean ± standard error).

Sprint data were log-transformed to meet the assumptions of ANOVA. The sprint speeds of the control lizards did not differ between sprint trials within each population ($P > 0.05$ in both cases)(Orford; trial 1, 1.1 ± 0.1, trial 2, 1.10 ± 0.09: Central Plateau; trial 1, 1.2 ± 0.11, trail 2, 1.18 ± 0.07). The gravid Orford lizards were significantly slower than the Orford control lizards ($F_{1,35}$= 5.279; $P < 0.05$), while gravid Central Plateau females were only marginally slower than the Central Plateau control females ($F_{1,47}$= 3.984; $P = 0.052$). Females from both populations ran significantly faster after parturition than before (CP: t statistic 4.291; $P < 0.001$; Orford: t statistic 4.203; $P < 0.001$) (Figure 8.2).

Regression analysis was used to further investigate factors responsible for the sprint performance of individual lizards within each population. The change in sprint speed for individual females (ie. gravid versus non-gravid) within each population was surprisingly unrelated to her burden, whether expressed as absolute mass or as a proportion of her body weight ($P > 0.05$ in all cases for both populations). There was also no relationship between the sprint speed while gravid and the burden of the clutch ($P > 0.5$ for both populations for relative burden and change in mass between trials).

I further investigated factors that may have affected the sprint speed of the burdened females. The regression of the change in weight (ie. the physical burden carried by each female) on body size (SVL) yielded residuals which were used as variates in a regression against sprint speed while gravid (see Sinervo, Hedges and Porter, 1991) (Figure 8.3). Residuals were calculated for each population from separate regressions. The females with the highest weight for their body length showed the slowest sprint speeds in both populations (Figure 8.4) (CP; r = 0.397; $P < 0.05$; Orford; r = 0.351; $P = 0.057$); however there was no significant relationship between the residuals from the first regression and the change in speed. Sinervo, Hedges and Adolph (1991) further calculated residuals from the regression of change in weight (burden) and the postpartum body mass. However, this is not possible with my data because there is no relationship between change in mass (burden) and postpartum body mass (CP; r = 0.098, P > 0.1; Orford; r = 0.085, P > 0.1).

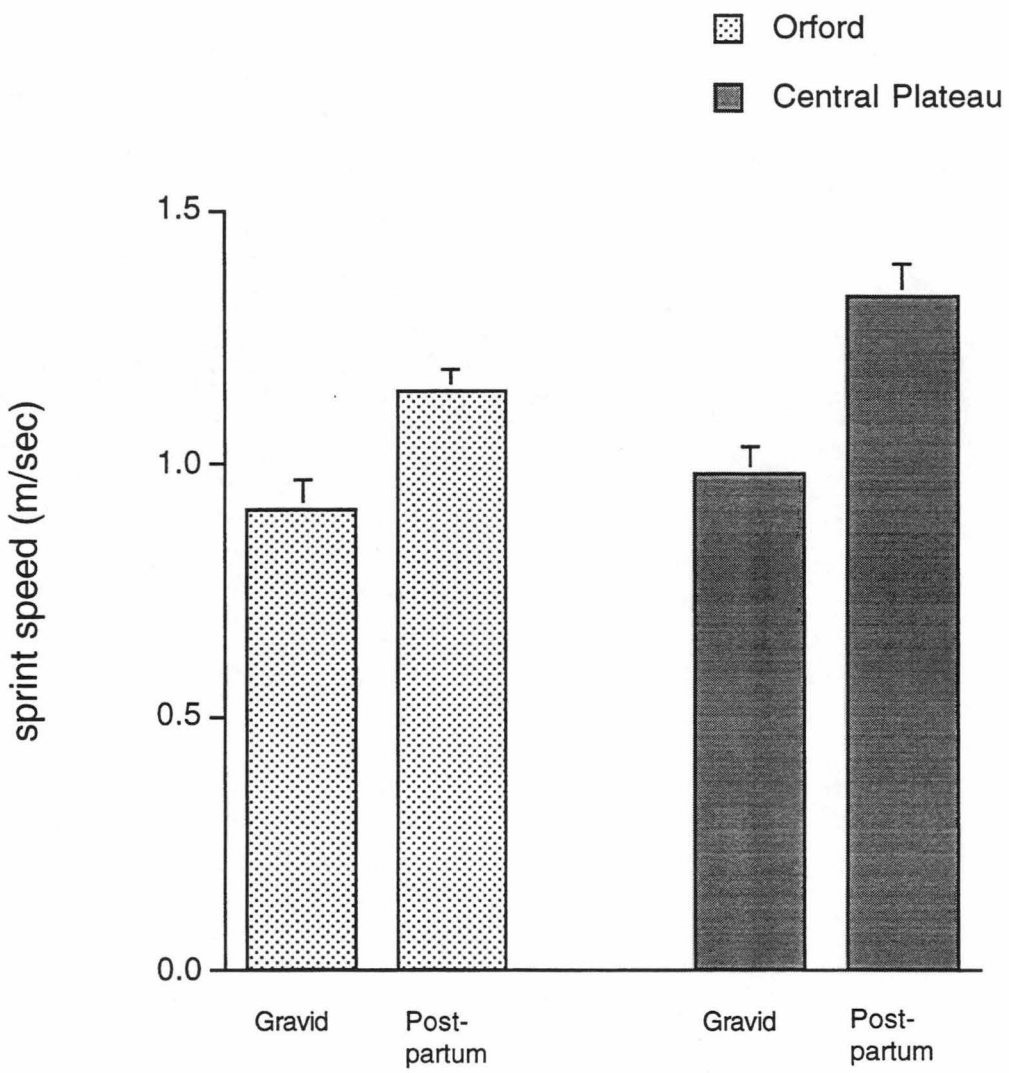

Figure 8.2. Sprint speed of female *Niveoscincus ocellatus* from Orford and the Central Plateau in gravid and postpartum states (mean ± standard error).

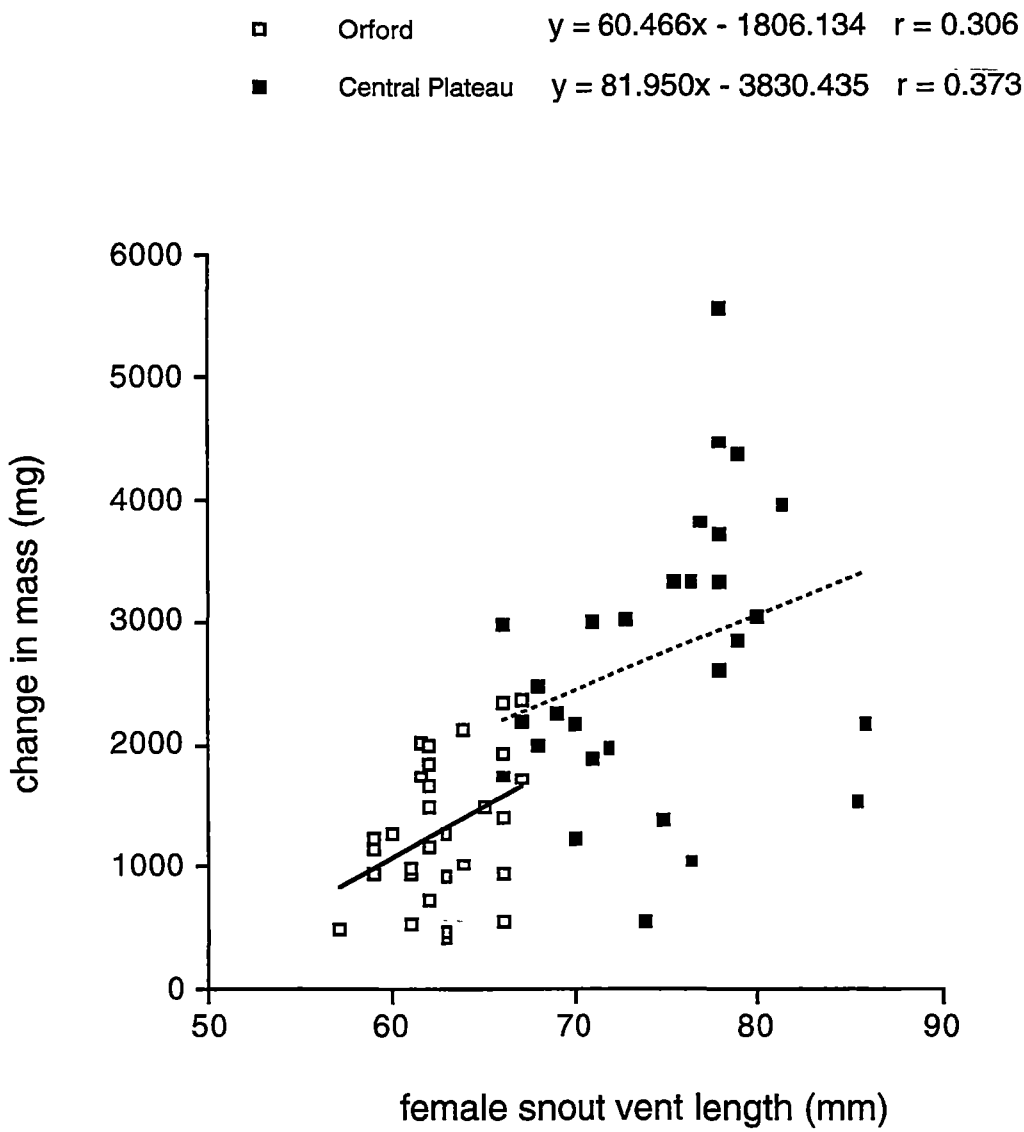

Figure 8.3. The relationship between female size and burden of clutch for female *Niveoscincus ocellatus* from Orford and the Central Plateau. Residuals from regression lines were used to analyse the effect of burden (corrected for body length) on sprint speed (see Figure 8.4).

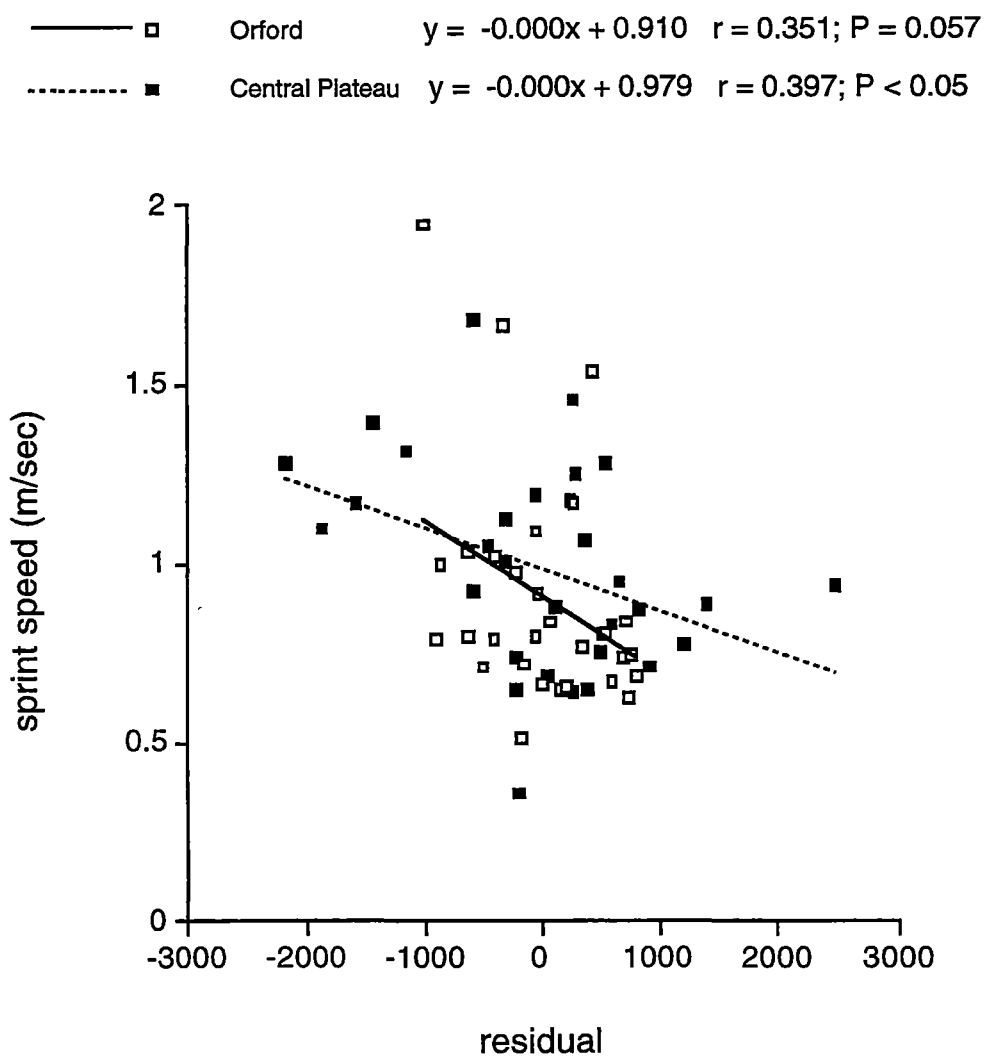

Figure 8.4. Regression of sprint speed of gravid *Niveoscincus ocellatus* from Orford and the Central Plateau and residuals of the regression of female size and burden (see Figure 8.3).

8.4 Discussion

Sprint speeds of female *N. ocellatus* were significantly reduced in the late stages of pregnancy compared to postpartum speeds. The magnitude of the reductions was about 20 % for the Orford population and 26 % for the Central Plateau population (Figure 8.2). This decrease is a potentially important cost to reproduction and survival because this species relies on flight, sometimes over considerable distance, to avoid capture or predation (pers. obs.). Furthermore, *N. ocellatus* is an active forager, actively searching for prey items and sometimes using rapid movement to capture prey (Wapstra and Swain, 1996). Costs to reproduction will be even more pronounced if there is a decline in endurance at the same time as a decrease in sprint performance (Cooper *et al.*, 1990). This was not tested in my study.

A decrease in sprint speed as a result of pregnancy has been documented in other skink species (Shine, 1980; Cooper *et al.*, 1990), other non-scincid species (Bauwens and Thoen, 1981; Van Damme, Bauwens and Verheyen, 1989; Sinervo, Hedges and Adolph, 1991), and other reptile species (Seigel, Huggins and Ford (1987). Shine (1980) demonstrated a decrease in sprint speed in the closely related species *Niveoscincus coventryi* (formerly *Leiolopisma coventryi*). The decrease in sprint performance appears to be of a similar magnitude in all of these studies. For example, Shine (1980) demonstrated a decrease of 20-30% in four species of skinks, Cooper *et al.* (1990) demonstrated a decrease of 27% in the skink *Eumeces laticeps*, and Sinervo, Hedges and Adolph (1991) found a decrease of between 20 and 45 % for different populations of the lacertid, *Lacerta vivipara*.

This present study has the advantage that it demonstrated a decrease in sprint performance not only relative to control (non-gravid females) lizards but also in individual females before and after parturition. Previous studies (Shine, 1980; Bauwens and Thoen, 1981; Sinervo, Hedges, and Adolph, 1991) have relied on only one of these comparisons.

It is clear that factors associated with pregnancy (presumably the increased physical burden of the clutch) confer a locomotory cost to the female. If the observed reduction in sprint performance is the result of the physical weight of the clutch, then is its magnitude related to the particular burden that an individual female carries? Preliminary examination of my data suggests that the decrease in sprint speed may indeed be related to burden; Central Plateau females have the largest relative burden and the largest decrease in sprint speed (26%). However, within each population there is no relationship between the change in sprint speed and individual burdens (either absolute load or load as a proportion of postpartum weight).

Evidence from other studies of the relationship between reproductive effort (burden or RCM) and the decrease in sprint performance is similarly inconclusive. Some previous studies (Shine, 1980; Seigel, Huggins and Ford, 1987; Van Damme, Bauwens and Verheyen, 1989) have found a relationship between individual sprint performance and RCM, while other studies (Brodie, 1989; Sinervo, Hedges and Adolph, 1991; Cooper *et al.*, 1990) have failed to demonstrate such a relationship.

The mechanism that decreases sprint speed in gravid lizards has not been identified. However, in this study there is evidence that a physical factor influenced sprint speed of gravid lizards. Although I found no relationship between sprint speed and burden (RCM), the females with the greatest burden for their body length experienced significantly greater impairment of their sprint speed while gravid. While the results should be interpreted with some caution ($P = 0.057$ for the Orford population), the consistency between the populations does argue for the decrease resulting from the physical burden. Sinervo, Hedges and Adolph (1991) likewise found that the decrease in sprint performance of gravid lizards was unrelated to the change in mass as a result of pregnancy, but found that in at least one population sprint speed was significantly related to the burden carried per unit body length. They attributed this relationship to anatomical interference of a large clutch and the ability of the female to run.

Most authors (e.g. Shine, 1980; Bauwens and Thoen, 1981; Madsen, 1987; Cooper *et al.*, 1990; Schwarzkopf, 1996a) suggest that a decrease in locomotor performance is likely to increase the risk of predation and/or reduce feeding opportunities. In laboratory trials, Shine (1980) tested this hypothesis directly with *N. coventryi*, and found that gravid females were more at risk of predation by snakes than males. However, Koufopanou and Bell (1984) argue that costs of reproduction measured in the laboratory may not reflect the costs in natural situations. An increase in the predation "risk" does not necessarily imply an increase in predation rate (Martin, 1996) because individuals may behave differently to cope with the temporary higher risk of predation.

Evidence from trials of predation on gravid female lizards in semi-natural conditions tends to confirm this assertion; gravid females did not suffer greater predation from snake and bird predators than non-gravid lizards in field enclosures (Schwarzkopf and Shine, 1992). As a result, Schwarzkopf and Shine (1992) conclude that impaired running speed by gravid female skinks (*Eulamprus tympanum*) may not translate into reduced survival, and that decrements in performance are probably not an important contributor to costs of reproduction. In natural situations females may reduce the potential costs of the reduction in locomotory ability by changing their behaviour. For example they may become more cryptic or alter defensive behaviour (e.g. Bauwens and Thoen, 1981; Brodie, 1989; Cooper *et al.*, 1990; Schwarzkopf and Shine, 1992, Braña, 1993).

Many authors have documented a reduction in feeding in gravid females (see Shine, 1980 for summary and more recently, Schwarzkopf, 1996a) which is thought to result from a decrease in mobility and thus feeding opportunities. Reduced mobility may be particularly costly to active forages (Schwarzkopf, 1996a). In this study, although there is a demonstrated difference in sprint ability, this does not appear to translate to a decrease in feeding rate (Wapstra and Swain, 1996). This may be because few of the food types in the diet (at least at the Orford study site) require sprint type behaviour to capture them. Furthermore, *N. ocellatus* displays a flexible approach to foraging (Wapstra and Swain, 1996)

suggesting that female lizards may be capable of shifting their foraging behaviour. Again, if changes in behaviour do occur, they do not significantly affect dietary composition (Wapstra and Swain, 1996).

Predation is an important selective force influencing morphology, behaviour, and life history tactics in natural populations (Curio, 1976). The adaptive nature of life histories is of central interest to evolutionary ecologists. Selection on RCM is under different selection pressures than egg or offspring size (Vitt and Price, 1982; Vitt, 1981). Whether the mass of the clutch is split among numerous smaller eggs or few larger eggs is influenced by advantages associated with offspring size and survival (see Chapter 4). The mass of the clutch, as a function of body mass and the ability of the lizard to carry that mass, is influenced by the probability of escape from predation and the ability to continue normal foraging behaviour (Vitt and Price, 1982). Selection operating on RCM is manifested by differential mortality and energetic costs of locomotion for gravid females (Vitt and Price, 1982).

In this study, the relative burden carried by females from the Central Plateau was larger than that of females from Orford (see also Chapter 4 for discussion of annual and site variation in RCM) and these females also exhibited the greatest reduction in sprint speed. However, observations suggest that this may not necessarily lead to a greater predation at the Central Plateau site. In the Central Plateau population it appears that adult survival is high (Chapter 5) and is an important selective force shaping reproductive effort. At the Central Plateau site, the large rock scree provides an abundance of cover and gravid lizards are rarely more than 50 cm from cover (pers. obs.). Furthermore, lizards are often seen basking under overhanging rocks, which effectively removes them from the view of aerial predators (birds). These factors may allow the females to have a relatively high reproductive effort for relatively low cost.

Further studies on the various costs associated with reproduction in small viviparous species are required to understand the selective pressures responsible for shaping reproductive effort. The RCM of a

species is a balance of the costs associated with its increase (increased predation rate or physiological costs) and factors that select for an increase in reproductive effort (e.g. the annual production of only a single, relatively large clutch, which is particularly common in viviparous species inhabiting high latitudes or altitudes (Tinkle, 1969)). There have been few studies that have examined survival costs associated with reproduction in natural situations (but see Madsen, 1987; Schwarzkopf, 1993), although many studies have suggested that survival costs are a factor in shaping reproductive effort. *Niveoscincus ocellatus* displays a reduction in sprint speed associated with pregnancy, yet there is no evidence that females experience a greater predation rate (Chapter 5), at either site, or a decreased feeding rate (Wapstra and Swain, 1996). Thus it is likely that other factors are also important in determining reproductive effort by females, including the energy available for reproduction, body shape and size, and the fecundity advantages that accrue from large clutches.

Chapter 9

General discussion

Life history patterns of reptiles differ widely between species and among populations of the same species (Tinkle, Wilbur and Tilley, 1970; Dunham, Miles and Reznick, 1988; Niewiarowski, 1994). Life history theory seeks to predict or test how traits such as number of offspring, size of offspring, and age at first reproduction vary among populations and species inhabiting different environments. Although geographic variation in North American iguanid species has been extensively studied, there have been relatively few intraspecific comparative studies of skinks. The limited range of studies restricts our ability to generalise about squamate life history, especially in regard to the importance of geographic variation. This thesis addresses some of these shortcomings by examining annual and geographic variation in life history traits in a common Tasmanian skink, *Niveoscincus ocellatus*, over a three year period within two geographically isolated populations.

A central question in life history research is whether variation between populations (or species) results from genetic variation or plastic responses to the environment. It is becoming increasingly clear that many patterns of geographic variation among lizard populations are primarily the result of proximate factors such as thermal conditions or food availability, rather than the previously untested assumption that differences represent evolved differences between populations. An understanding of the role of proximate influences on variation in life history traits (phenotypic plasticity) is important for a complete understanding of life history patterns (Ballinger, 1983; Caswell, 1983; Stearns and Crandall, 1984; Sinervo and Doughty, 1996).

Common garden type experiments and reciprocal transplant experiments are relatively new methods in herpetology, and are increasingly being used to separate ultimate (genetic differences) and proximate (phenotypic

plastic responses) sources of variation in life history traits between species or populations. Reptiles, however, are an ideal group with which to use this type of approach to untangle the complex relationships between genetic and environmental determinants of phenotype and fitness. It is, therefore, perhaps surprising that the majority of these types of investigation on lizards have been restricted to the genera *Sceloporus* and *Lacerta*.

Niveoscincus ocellatus occurs over a wide climatic range and over this range displays considerable variation in life history traits. This species is well suited for an examination of possible causes of life history variation. The populations I chose for study are genetically isolated and are separated by a distance of approximately 200 kilometres with no continuous distribution between them. Furthermore, Melville (unpublished data) recently showed that there is some genetic divergence between the populations. Differences between the populations could therefore be interpreted as either evolved differences due to selective pressures over time at each of the sites, or as the result of proximate effects such as temperature or food availability.

Variation in life history traits in *N. ocellatus* was found at two levels: geographic variation between populations and annual variation within populations. While inter-annual variation within the same population of long-lived species can only result from phenotypic plastic responses to environmental variation, geographic variation in life history traits may be interpreted in terms of evolved genetic differences or proximate environmental effects or a combination of these. The clear evidence for inter-annual variation found in both populations of *N. ocellatus* studied raises the possibility that differences between populations may also be the result of phenotypic plasticity in response to local environmental conditions.

Field studies correlating environmental conditions with variations in observed life history traits are crucial in understanding variation in life history traits (Ballinger, 1983, Ford and Seigel, 1989b); however, the number of uncontrolled variables makes it difficult to quantify the

specific factors which influence differences. Controlled experimental studies may be useful in isolating the role of phenotypic plasticity and the specific factors involved in causing the variation (e.g. Kaplan, 1987; Ford and Seigel, 1989a). In the third section of this thesis I investigated the role of phenotypic plasticity in variation in life history traits of *N. ocellatus*. Specifically, I investigated the influence of the thermal environment (through its effects on basking and activity) on life history variation and its possible role in explaining annual and geographic variation.

Offspring size and postnatal growth rate were used as focal points for investigation because of the important ramifications of these traits for the entire life history strategy of an individual. In Chapter 5, I showed that the Orford and Central Plateau populations displayed different growth patterns. I hypothesised that reduced growth rates at the Central Plateau were responsible for a delay in reproduction, as a result of which these lizards continued to grow for another season reaching a larger size at sexual maturity. In Chapter 6, I investigated whether growth rate at the study sites was a reflection of genetic differences between populations or a consequence of phenotypic responses to the thermal environment. I used two approaches: a common garden experiment in the laboratory and a reciprocal transplant experiment involving two sets of outdoor enclosures.

The growth rate of juvenile *N. ocellatus* is positively correlated to basking opportunities in the laboratory and to thermal conditions in field enclosures, but was not influenced by any underlying genetic differences between the populations. Although there is now a growing body of research into sources of variation in growth rate of juvenile lizards, particularly in species that display geographic life history variation, consistent patterns are yet to emerge. While all studies to date have documented a large phenotypic plastic component in growth rate, the evidence for a genetic component to differences between populations is less conclusive. Growth rate may be expected to evolve between populations that have been isolated for long periods, or in which there are strong selective pressures on growth rate. I suggest that growth rate in *N. ocellatus* has not evolved in response to selective pressures at the

study sites because growth rate is optimised at each site through phenotypic plastic responses of growth rates to environmental variability.

Growth rate is a widely studied life history trait because of its effect on other important life history characteristics. For example, I demonstrated that even small differences in growth rate prior to maturity may be responsible for the difference in adult body sizes at Orford and the Central Plateau. Recently, Adolph and Porter (1996) proposed that geographic variation in size and age at maturity in lizards occupying different climates may be explained by differences in growth rates between populations as a result of proximate effects of the thermal environment. The findings of my study are consistent with the predictions from their model: populations in cold environments are predicted to mature later and at a larger size. The differences in size and age at maturity in *N. ocellatus* at the two study sites are consistent with life history strategies that maximise lifetime fecundity. A cost to fecundity in early life because of delayed maturity at the Central Plateau is outweighed by fecundity gains later in life through increased body size (fecundity in *N. ocellatus* is tightly coupled to female size) and high adult life expectancy. The alternative strategy of maturing at a smaller size at Orford is coupled with earlier maturity and lower adult life expectancy.

The life history patterns exhibited by *N. ocellatus* at the Central Plateau and at Orford are consistent with most of the predictions from the Adolph and Porter (1993) model. As in their later model (Adolph and Porter, 1996), life history variation between populations is explained in terms of the effect of the thermal environment on daily and seasonal lizard activity. They predicted that individuals from populations with short potential activity seasons (e.g. Central Plateau) will have a high annual survival, a low annual reproductive output, delayed maturity at a larger size. In contrast, individuals with long activity seasons (e.g. Orford) are expected to mature earlier at a smaller size, have a lower annual survival, and have a lower reproductive output. My findings did not conform to the predicted reproductive output. Reproductive output at the Central Plateau is generally the same as at Orford in relative terms

(RCM) and is higher is absolute output (clutch mass and number of young).

I suggest that high reproductive output is possible at the Central Plateau because (1) a high reproductive output does not severely decrease survival of female lizards at this site, and (2) there is adequate energy available (food and foraging opportunities) to permit a relatively high reproductive output. Further work to identify factors that influence reproductive output between populations (and between years within populations) of skinks is required because to date a variety of patterns have been identified. For example, Forsman and Shine (1995) found that reproductive output was greater in cold southern populations of *Lampropholis delicata* than in populations from warmer climates, whereas Rohr (1997) found that in *Eulamprus tympanum* alpine populations had a lower reproductive output than lowland populations.

The thermal environment has profound effects on the life history of *N. ocellatus*. In addition to the effects of the thermal environment on growth and its implications on size and age at maturation, the thermal environment also influences gestation length. In the laboratory, gestation length was affected by the basking opportunities available to females. The major implication of this finding, apart from its possible effects on the timing of reproductive cycles, was the influence of gestation length on juvenile morphology and growth rate. Juvenile fitness (size, condition and growth rate) is maximised through short gestation. These findings may be used, in part, to explain geographic variation in offspring phenotype between years, and also have implications for the timing of reproductive cycles in different climatic regions. Variation in the size of offspring is often explained in terms of selective pressures acting on offspring size. However, in my study two other factors were found to influence the size of individual offspring: the thermal environment through its influence on maternal basking behaviour, and the trade-off between number and size of young mediated by energy availability to individual females.

In *N. ocellatus* flexible timing of reproductive cycles is undoubtedly responsible, in part, for their widespread distribution over a range of climatic conditions. Reproductive events (ovulation, parturition and spermatogenesis) at the Central Plateau occur approximately one month later than at Orford. This strategy ensures that gestation in the Central Plateau population occurs relatively rapidly during the warmer summer months which maximises offspring fitness. Work on other widespread species has similarly documented variation in the timing of reproductive cycles that is thought to account for the widespread geographic and elevational distribution of these species (e.g. *Anolis sagrei*, Lee *at al.*, 1989; *Sceloporus scalaris*, Mathies and Andrews, 1995).

Niveoscincus ocellatus maintains an annual reproductive cycle in which 100% of females reproduce each year across its range. Within the same genus, the alpine specialist species *N. greeni* and *N. microlepidotus* exhibit a rare (for lizards) biennial reproductive strategy in which embryos are carried over winter, presumably because thermal conditions do not allow gestation to be completed within one season. Although *N. ocellatus* occurs sympatrically with these species at the lower boundary of their altitudinal distributions, it does not extend to the higher altitudes which are the domain of the alpine species. I suggest that one factor that may ultimately limit the distribution of *N. ocellatus* is its reproductive pattern which is geared to producing viable offspring within a single activity season.

In this study, I have demonstrated that much of the annual and geographic variation in life history traits results from phenotypic plastic responses to environmental variables, particularly the thermal environment. Species such as *N. ocellatus* that occur in a variety of thermal environments demonstrate one possible advantage of having a phenotypically plastic life history. Indeed, widespread species that are not genetically restricted to certain thermal conditions may be better able to cope with temporal or spatial climatic change. The evolution of distinct local genotypes may be unnecessary if phenotypic plasticity is sufficient to induce variation in life history traits to suit particular environments. Furthermore, it may be difficult for natural selection to optimise traits,

even under strong and consistent selective pressures, if relatively little of the variance in traits is due to genetic factors (Shine, 1995).

This thesis provides important comparative data on *Niveoscincus ocellatus*. A valuable extension of my study would be an investigation of the generality of its findings, to other populations of the same species, to other members of the genus, particularly those that occupy wide geographic ranges, and to other geographically widespread skink species. Clearly, although some general patterns in life history variation among squamate reptiles are emerging, further studies of a range of species from different habitats and climates from a variety of taxa are required. Such studies should focus on relating variation in life history traits of a species to broader ecological parameters to understand the possible adaptive significance of the variation observed.

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